The Ecological Factors that Structure the Composition and Function of Saprotrophic Fungi: Observational and Experimental Approaches

by

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DEDICATION

To Van Cline, for instilling in me a love and curiosity for the natural world.

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ABSTRACT

The Ecological Factors that Structure the Composition and Function of Saprotrophic Fungi: Observational and Experimental Approaches

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A central goal in ecology is to understand the processes underlying the distribution and abundance of species at local, regional and global scales. As mediators of biogeochemical cycles, understanding the mechanisms that govern microbial community assembly are of ecosystem-scale significance, yet they remain a critical gap in our ecological knowledge. Through a combination of observational and experimental approaches, I explored the roles of selection, drift and dispersal in structuring microbial communities of saprotrophic fungi at a variety of spatial and temporal scales. Furthermore, I investigated the link between fungal community composition and functional characteristics in order to understand whether the factors that structure microbial community assembly have direct consequences to ecological function. First, I explored the role of selection in structuring soil microbial community assembly along a secondary successional chronosequence. My work revealed that the accumulation of organic matter and change in plant litter biochemistry during plant succession (10 to 86 yrs following agricultural abandonment)

shaped saprotrophic fungal composition and their physiological potential to metabolize plant detritus, providing support for the idea that changes in plant communities have direct outcomes on the competitive dynamics of saprotrophic microbial communities in soil. Secondly, I found that both dispersal limitation and drift had a persistent effect on the phylogenetic structure and functional richness of saprotrophic fungal communities across a long-term glacial chronosequence (9,500 to 13,500 yrs following glacial retreat). Last, I manipulated initial colonizers and leaf litter biochemistry to understand the relative importance of priority effects and selection in structuring saprotrophic fungal communities and leaf litter decay. I found that the strength of priority effects was dependent on leaf litter biochemistry and physiological traits within a regional species pool. Together, these findings demonstrate that selection, drift and dispersal structure fungal community assembly at both local and regional levels with important consequences to plant litter decay. Furthermore, my observations provide support for the assertion that the same ecological forces structuring plant and animal communities also shape the assembly of saprotrophic fungi.

CHAPTER I

Introduction

A central goal in ecology is to understand the processes underlying the distribution and abundance of species at local, regional, and global scales. Understanding these patterns may be critical to predicting ecological responses to anthropogenic change. However, because primary mechanisms of community assembly depend on the organism, as well as temporal and spatial scale under consideration, we have historically lacked a single framework that unifies all ecological and evolutionary processes contributing to the generation and maintenance of species diversity. For example, the small size and rapid generation time of microorganisms has distinguished microbial community assembly from the processes governing the biogeography of plants and animals (Martiny et al. 2006). To address the need for a conceptual synthesis of community ecology, a unified theory was proposed, thereby condensing the factors influencing community assembly intro four ecological and evolutionary processes: selection, drift, speciation, and dispersal (Vellend 2010). Due to its simplicity, this framework can be applied to investigating mechanisms of community assembly across all domains of life, including microorganisms (Nemergut et al. 2013). However, understanding the mechanisms that govern the most abundant and diverse organisms on Earth remains a substantial gap in our ecological knowledge. Using Vellend's framework, my dissertation investigates the relative importance of selection, dispersal, and drift in structuring microbial communities across a range of spatial and

temporal scales. Further, I explore the link between microbial composition and the decay of plant detritus in order to understand whether the ecological factors that structure soil microbial communities have direct consequences on ecological function, and, ultimately, to ecosystem-scale processes.

A unified theory of community assembly

At local, regional, and global scales, community assembly can be attributed to the processes of selection, drift, speciation, and dispersal (Vellend 2010). This framework was adapted from foundational principles of population genetics, considering changes in taxa abundance, rather than allele frequencies. For the purposes of this dissertation, I define a community as a group of organisms in a specified location. Selection describes fitness differences between individuals that arise from trait variation occurring when an individual reproduces or replicates at a higher rate relative to others. Ecological drift occurs when stochastic dispersal, death, and reproduction alter species abundance. Diversification (modification of Vellend's model *sensu* Nemergut et al. 2013) results from genetic mutation and the generation of novel genotypes within a community. Finally, dispersal is the transportation and successful establishment of propagules from one location to another. Under this framework, diversification, dispersal, and drift can enhance diversity; whereas, selection and low dispersal can limit local richness by constraining organisms in the regional species pool from establishing in a particular location. The inclusion of diversification is a major strength of Vellend's conceptual model, which others have not considered, and yet remains an important mechanism of community assembly (Rainey et al. 1998). Furthermore, Vellend's framework recognizes the dual importance of deterministic factors, driven by fitness differences arising from species traits, as well as stochastic forces.

Due to its synthetic nature, many important mathematical and conceptual models can be captured within Vellend's framework of community assembly. Following the idea that species traits determine outcomes of interactions (*e.g.*, competition and predation), the process of selection is similar to the contemporary concept of the ecological niche (Chase and Leibold 2003). Therefore, coexistence models that link community composition to abiotic conditions through species traits, including theoretical and empirical tests of life history tradeoffs (Tilman 1994, Cadotte et al. 2006), fall under the umbrella of selection. Island biogeography theory captures relationships between dispersal and selection (MacArthur and Wilson 1967); whereas, neutral theory models community assembly by considering the roles of dispersal and drift (Hubbell 2001). As a result of priority effects, theories of community assembly history explicitly consider the importance of the order and timing of stochastic dispersal on consequences of selection and community assembly (Wilbur and Alford 1985, Fukami et al. 2005). From four distinct perspectives, the metacommunity framework considers selection, dispersal, and drift to model spatially explicit community dynamics (Holyoak 2005). Importantly, by incorporating a historical perspective, Ricklefs and Schluter (1993) consider the importance of diversification and past selection on community patterns across broad spatial and temporal scales. The intended breadth of this framework provides utility to microbial ecologists, because major processes governing community assembly across temporal and spatial scales remains largely unknown.

Characteristics unique to microorganisms influence community assembly

Characteristics unique to microorganisms may enhance or diminish the importance of selection, drift, diversification, and dispersal relative to the assembly of other biotic communities (Nemergut et al. 2013). For example, due to the small size of microorganisms, passive transport is the prevalent mode of dispersal (but see Trail 2007) and enables propagules to travel great

distances (Hallenberg and Kuffer 2001, Favet et al. 2012). Yet, despite the potential for long-distance dispersal events and microbial cosmopolitanism (Finlay 2002), mounting evidence indicates that microbial communities are geographically isolated (Norros et al. 2012, Peay et al. 2012). Furthermore, the combination of dispersal limitation and short generation times may enhance the roles of drift and diversification in structuring microbial communities (Papke and Ward 2004). Because a majority of individuals in a microbial community lie dormant, these organisms persist until more favorable environmental conditions arise (Lennon and Jones 2011). Thus, dormancy maintains microbial diversity, as inactive organisms function as a 'seed bank' (Jones and Lennon 2010, Lennon and Jones 2011). The transfer of genetic material between different taxa (horizontal gene transfer) is also prevalent among Bacteria and Archaea, indicating that selection may occur at the level of functional genes and not at a taxonomic level (Gogarten and Townsend 2005, Burke et al. 2011). Furthermore, as the most abundant and diverse organisms on Earth, functional overlap between microbial taxa (Talbot et al. 2014) could reduce the importance of selection in shaping community composition, resulting in lasting effects of historical contingencies (Fukami et al. 2010, Eisenlord et al. 2012).

Regardless of these unique characteristics, microbial communities share several common biogeographical patterns with macrobial communities (Martiny et al. 2006). For example, the rank abundance of microbial taxa follows an exponential decay model, in which few taxa are present in high abundance and the majority of organisms are rare (McGill et al. 2007, Nemergut et al. 2013). Following the established species-area relationship observed in many plant and animal communities, microbial taxa richness increases with geographic area as a power function (Green and Bohannan 2006, Nemergut et al. 2013). Furthermore, a review of distance-decay relationships of microbial communities indicates that a combination of contemporary selection

and historical processes, including drift and past selection, structure microbial community assembly (Hanson et al. 2012). These patterns demonstrate that ecological and evolutionary processes unquestionably govern microbial community assembly; however, elucidating the mechanisms behind these patterns remains unresolved (Hanson et al. 2012, Nemergut et al. 2013).

Potential links between microbial community composition and function

Because microorganisms perform fundamental biological processes that govern biogeochemical cycles, understanding the functional consequences of microbial community assembly is of ecosystem-scale significance. Microorganisms vary in their capacity to degrade components of plant litter, as supported by the analysis of individual bacterial genomes and the resource use of active litter-decay fungi (McGuire et al. 2010, Berlemont and Martiny 2014). However, large overlap in functional characteristics within fungal communities has been observed, indicating a high degree of functional equivalence between relatively different organisms and the communities they compose (Hättenschwiler et al. 2005, Talbot et al. 2014). To investigate the link between composition and function, I proposed to quantify the genetic and enzymatic potential of microbial communities to degrade components of the plant cell wall (Figure 1.2, Zak et al. 2006) and compare to the composition of saprotrophic soil communities. These organic substrates are decomposed through the production of extracellular enzymes, encoded by genes within each microbial genome. Therefore, by investigating the relationship between the abundance and composition of genes encoding enzymes ('genetic potential'), as well as potential extracellular enzyme activity of microbial communities, we can begin understanding the complex linkages between microbial community composition, microbial functional potential, and rates of litter decay. In my dissertation, I sought to understand whether

factors structuring community assembly have direct consequences to soil biogeochemical processes.

Preview of chapters

Through a combination of observation and experimental approaches, I explored the importance of selection, dispersal and drift in structuring soil microbial community composition and function across a variety of spatial and temporal scales. Furthermore, molecular tools enabled me to quantify microbial community composition and genetic potential at a fine resolution using culture-independent methods. To elucidate mechanisms of microbial community assembly, I used saprotrophic fungi as a model system due to their ecological significance in metabolizing the lignified components of plant detritus (Berg and McClaugherty 2008), as well as their practical ease of manipulation. In Chapter II, I explored the role of selection in structuring soil microbial community assembly along a secondary successional chronosequence. My work revealed that the accumulation of organic matter and change in plant litter biochemistry during plant succession (10 to 86 yrs following agricultural abandonment) shape saprotrophic fungal composition and their physiological potential to metabolize plant detritus, providing support for the idea that changes in plant communities have direct outcomes on the competitive dynamics of saprotrophic microbial communities in soil. In Chapter III, I found that historical processes (*i.e.*, the combination of dispersal limitation and ecological drift) had a persistent effect on the phylogenetic composition and functional diversity of saprotrophic fungal communities across a long-term glacial chronosequence (9,500 to 13,500 yrs following glacial retreat). In Chapter IV, I manipulated leaf litter biochemistry and initial colonization to understand the relative importance of priority effects and selection in structuring saprotrophic fungal communities and decomposition dynamics. I found that that the strength of priority effects was

dependent upon features of habitats and physiological traits within a regional species pool. Together, these findings demonstrate microbial community assembly is dependent upon competitive dynamics at the local level, as well as historical contingencies across broad spatial and temporal scales. Furthermore, these observations provide strong support that the same ecological forces that structure plant and animal communities also shape the assembly of saprotrophic fungal communities.

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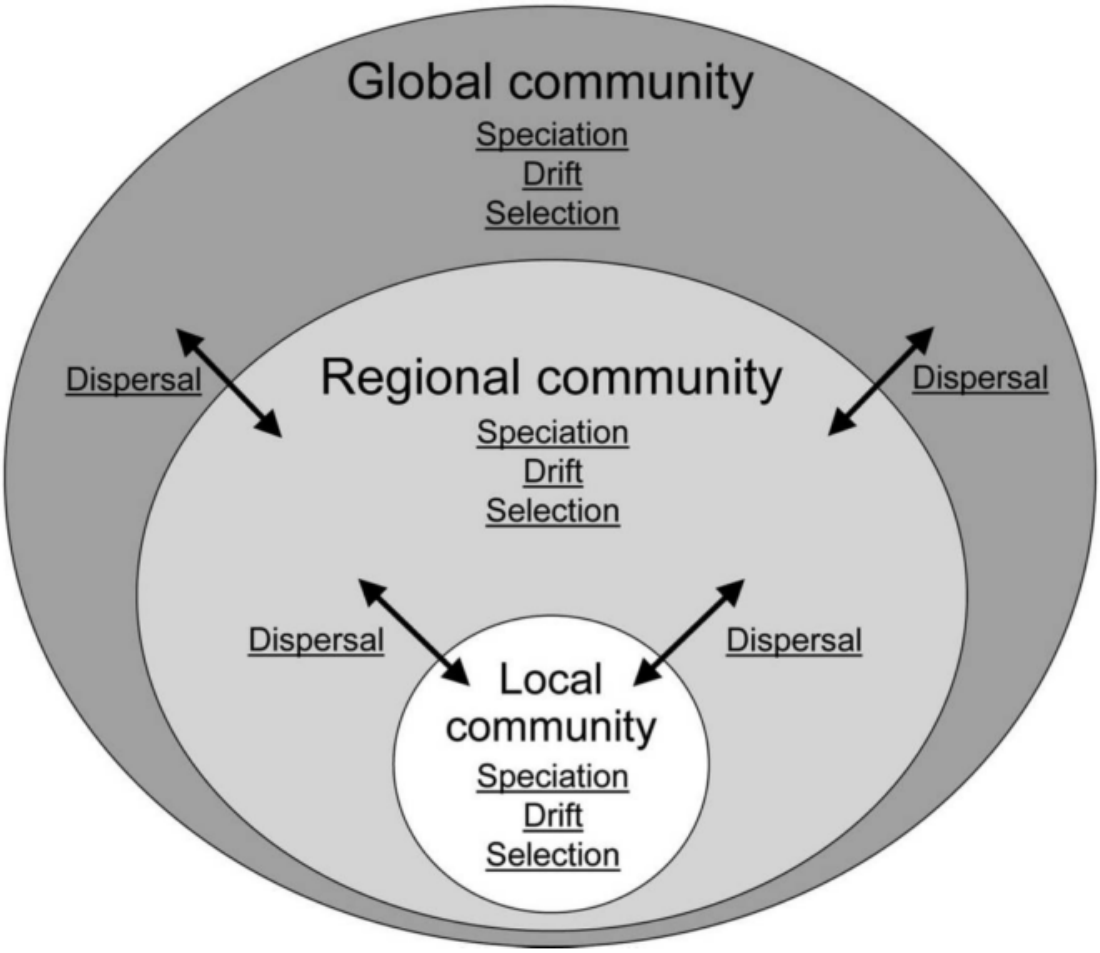

Figure 1.1. Conceptual model of community assembly (Vellend 2010). Four processes structure community composition across local, regional and global scales, including selection, drift, speciation and dispersal.

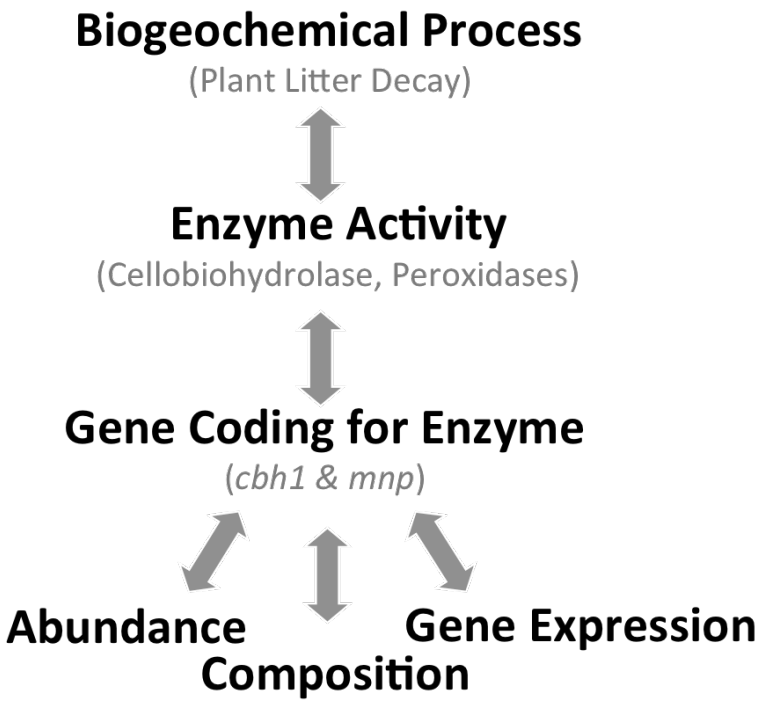

Figure 1.2. Conceptual diagram linking functional genes and the biogeochemical process of plant litter decay through the activity of extracellular enzymes. The abundance, composition and expression of functional genes regulate the production of extracellular enzymes, thereby mediating the process of decomposition. Adapted from Zak et al (2006).

CHAPTER II

Resource availability structures soil microbial composition and function across an old-field chronosequence

ABSTRACT

Although we understand the ecological processes eliciting changes in plant community composition during secondary succession, we do not understand whether co-occurring changes in plant detritus shape saprotrophic microbial communities in soil. In this study, we investigated soil microbial composition and function across an old-field chronosequence ranging from 16 to 86 years following agricultural abandonment, as well as three forests representing potential late-successional ecosystems. Fungal and bacterial community composition was quantified from ribosomal DNA, and insight into the functional potential of the microbial community to decay plant litter was gained from shotgun metagenomics and extracellular enzyme assays. Accumulation of soil organic matter across the chronosequence exerted a positive and significant effect on fungal phylogenetic β-diversity and the activity of extracellular enzymes with lignocellulolytic activity. In addition, the increasing abundance of lignin-rich C4 grasses was positively related to the composition of fungal genes with lignocellulolytic function, thereby linking plant community composition, litter biochemistry, and microbial community function. Edaphic properties appeared to be the primary agent shaping bacterial communities, as bacterial β-diversity and functional gene composition displayed a significant and positive relationship to

soil pH across the chronosequence. The late-successional forests were compositionally distinct from the oldest old fields, indicating that substantial changes occur in soil microbial communities as old fields give way to forests. Taken together, our observations indicate that resource availability in the form of litter biochemistry shapes saprotrophic fungal composition and function during secondary succession and provides support for the idea that soil microbial communities change in parallel with plant communities during secondary succession.

INTRODUCTION

Ecological succession describes changes in biotic community composition following a disturbance (Connell and Slatyer 1977). Although mechanisms causing changes in plant community composition during secondary succession have received much attention over several decades (*e.g.,* Grime 1979, Tilman 1988), we do not understand the extent to which saprotrophic soil microbial communities change through time. Because plant species differ in the production and biochemical composition of detritus, changes in plant community composition during secondary succession could modify the nature of organic substrates which structure saprotrophic soil microbial communities (Paul and Clark 1990, Martiny et al. 2012). If soil microorganisms possess ecological trade-offs analogous to those in plant communities, then changes in the availability of growth-limiting substrates (*i.e.,* plant detritus) for saprotrophic metabolism should cause soil microbial communities to change during the course of secondary succession due to competitive displacement.

Changes in the functional characteristics of plant communities during secondary succession have the potential to exert selective pressure on microbial communities in soil by altering the availability of growth-limiting substrates (Bardgett et al. 2005). Plant litter is biochemically heterogeneous, comprised of simple organic molecules (*e.g.,* saccharides, amino acids, and nucleic acids), cellulose, hemicellulose as well as polyphenolic compounds such as lignin. Whereas bacteria and molds are more competitive extracting energy from mono- and polymeric sugars (Hudson 1968), the ability to degrade lignin is mainly conserved in the fungal subkingdom Basidiomycota (Baldrian 2006). Because a limited number of microorganisms are capable of degrading this polyphenolic molecule, lignin regulates litter decomposition rates and alters microbial composition during decay (Talbot et al. 2011, McGuire et al. 2010). Increasing

litter lignin:N in a grassland chronosequence indicates a decline in resource availability for saprotrophic microorganisms (Knops and Tilman 2000, Quested et al. 2007) and provides a mechanism by which plants drive concomitant changes in soil microbial communities. If lignified plant detritus increases during secondary succession, it should favor organisms with the physiological capability to metabolize these substrates, thereby altering the composition and function of soil microbial communities.

While it is well established that microbial biomass, respiration, and net N mineralization increase during secondary succession (Zak et al. 1990, Waldrop et al. 2006), advances in molecular ecology enable us to characterize microbial composition and function across temporal scales. Recent evidence revealed that soil bacterial communities can be influenced by geologic substrate age, soil properties, as well as and plant community composition (Nemergut et al. 2007, Jangid et al. 2013). Despite their key functions in soil C and N cycling, understanding of changes in fungal communities during plant succession lags farther behind (Jumpponen 2003, Cutler et al. 2014). Inasmuch, we have a limited understanding of the mechanisms that might link changes in plant and microbial communities in soil across temporal and spatial extents.

To test these ideas, we investigated bacterial and fungal composition and functional potential across a series of nine old fields, ranging from 16 to 86 years since agricultural abandonment, as well as three adjacent forests representing potential late-successional ecosystems. We hypothesized that differences in soil microbial communities would be structured by changes in resource availability, a consequence of changes in biochemistry of plant detritus during secondary succession. If the succession of microbial communities is related to shifts in plant functional traits that influence the biochemistry of detritus, we expect that microbial functional potential will co-vary with microbial communities across the chronosequence. We

quantified fungal and bacterial richness, as well as phylogenetic β-diversity in an established chronosequence with well-documented changes in plant community composition. Additionally, we used shotgun metagenomics and extracellular enzyme assays to quantify the functional potential of saprotrophic communities to degrade components of plant detritus.

METHODS

Study sites

We studied soil microbial communities in an old-field chronosequence located at the Cedar Creek Ecosystem Science Reserve (Bethel, MN). The landscape was formed following the Wisconsin glacial retreat (*ca.* 12000 B.P); mean annual temperature is 6 °C with annual precipitation of 66 cm. Nine fields were selected from 21 potential sites (Knops and Tilman 2000), all occurring on sandy glacial outwash with similar soil development. We also sampled three forests in this landscape, including an oak savanna (OS), an upland pin oak forest (UPO; *Quercus ellipsoidalis*), and a northern hardwood (NH) forest, residing on the same soil parent materials as the old fields. Differences in plant community composition between late-successional ecosystems primarily result from fire frequency and its influence on soil N availability (Zak et al. 1990).

Soil sampling

In each old field, we collected soil cores (3-cm diameter) to a depth of 5 cm from 20 randomly selected quadrats across two previously established transects (Knops and Tilman 2000). In the forests, we established two parallel 40-m transects and randomly sampled at 10 points along each transect. Within each old field and forest, soil samples were composited and sieved (2 mm) to homogenize soil and remove roots. A 2-g subsample was removed for enzyme

analysis and it was stored at 4° C (Burns et al. 2013). Remaining soil samples were immediately frozen and transported to the University of Michigan for molecular analyses.

Environmental characteristics

Soil and root characteristics were quantified in old fields and forests; whereas, aboveground plant biomass was quantified in the nine old fields (Table S2.1). A soil subsample from each old field and forest was sent to University of Wisconsin Soil Laboratories (Verona, WI) to quantity soil pH, organic matter (SOM), and total N. Briefly, soil pH was quantified in deionized water using a pH meter (ThermoFischer Scientific). Organic matter was determined using a Leco CNS2000 Analyzer (LECO® St. Joseph, MI). Total soil N was measured colorimetrically following digestion in concentrated H_2SO_4 (Lachet Instruments, Loveland, CO). Root biochemistry was characterized by lignin, cellulose, hemicellulose, and total N. Root lignin was determined by the acid detergent lignin (ADL) procedure, in which ADL is determined gravimetrically as the residue remaining upon ignition after H_2SO_4 treatment (Goering & Van Soest, 1970). Root cellulose was calculated by subtracting percent acid detergent fiber (ADF) and lignin from root dry mass. ADF was determined gravimetrically as the residue remaining after dissolution and extraction of cell solubles, hemicellulose and soluble minerals with hexadecyltrimethylammonium bromide and sulfuric acid. Hemicellulose was determined by subtracting ADF and neutral detergent fiber (NDF; Van Soest et al. 1991). In old fields, biomass of each plant species was measured by clipping and drying all aboveground plant material. We assigned plant species to C3 grasses, C4 grasses, forbs, legumes, sedges and woody plants and calculated relative dominance of each functional group.

DNA extraction & community analysis

Using MoBio PowerMax Soil DNA Extraction kit, total DNA was extracted from three replicate samples, removed from composite soil samples collected in each old field and forest. DNA was extracted from 5-g of soil and stored at -80 °C until we initiated metagenome sequencing and PCR amplification. Targeted amplification of bacterial and fungal ribosomal genes was performed to characterize the composition of soil microbial communities. We analyzed fungal composition by targeting the 28S gene using primers LROR_F (5′-CCGCTGAACTTAAGCATATCAATA-3′; Amend et al. 2010) and LR21 (5'-ACTTCAAGCGTTTCCCTTT-3'; Hopple & Vilgalys 1994). Fungal primers were selected to preferentially amplify Basidiomycota, organisms largely responsible for lignin metabolism in soil. To quantify bacterial community composition, the 16S ribosomal gene was targeted using primers 27f (5'-AGAGTTTGGATCMTGGCTCAG – 3') and 519r (5'-GWATTACCGCGGCKGCTG-3'; Lane et al. 1991). Triplicate 28S PCR reactions for each composite soil sample contained 1 µL of DNA, 0.5 µL of 20 µM forward primer, 0.5 µL of 20 µM reverse primer, 2.5 µL dNTPs (2 µM), 2.5 µL 10X PCR buffer (1.5 mM $MgCl_2$; Roche, Hamburg, Germany), 1 µL BSA, 0.5 µL high-fidelity *Taq* polymerase (Roche, Hamburg, Germany), and 16.5 µL molecular grade water. Following an initial denaturation step at 95 °C for 10 min, fungal PCR was cycled 34 times at 95 °C for 1 min, 54 °C annealing temperature for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 7 min. Negative controls were included in every PCR reaction. Triplicate 16S PCR reactions contained 1 µL of DNA, 0.5 µL of 20 µM forward primer 27f, 0.5 µL of 20 µM reverse primer 519r, 0.5 µL dNTPs (20 mM), 5.0 µL 10X PCR buffer (1.5 mM $MgCl_2$; Roche, Hamburg, Germany), 0.6 µL high-fidelity *Taq* polymerase (Roche, Hamburg, Germany), and 16.9µL molecular grade water. Following an initial

denaturation step at 95 °C for 10 min, PCR was cycled 26 times at 94 °C for 30 s; we used a 55 °C annealing temperature for 1 min, 72 °C for 90s, and a final extension at 72 °C for 20 min.

Sequencing was performed on a PacBio-RS II system utilizing circular consensus technology, which can generate 99.5-99.9% sequence accuracy for DNA fragments ranging from 150 to 500 bp (Travers et al. 2010). PCR products were purified and quantified using Qiagen MinElute PCR and PicoGreen dsDNA kits. Two barcoded samples, pooled in equimolar concentration were multiplexed per SMRT chip. Twelve SMRT chips were sequenced at the University of Michigan Sequencing Facility. Sequences were processed in Mothur using established pipeline procedures (Schloss et al. 2011). Fungal and bacterial sequences were each rarefied to 5,919 sequences per site, according to the site that yielded the fewest number of sequences, to ensure equally sampling across all old fields and forests. Sequences were sorted by barcode and trimmed, removing primers and barcodes, followed by alignment to 16S and 28S reference alignments (James et al. 2006, Quast et al. 2013). DNA contaminants (chloroplast, mitochondrial and unknown DNA) and chimeras, identified using uchime (Edgar et al. 2011), were removed from downstream analysis. Operational taxonomic units (OTUs) were clustered at 97% (16S) and 99% (28S) sequence similarity according to established procedures to target 'species' designations of bacteria and fungi (Schloss et al. 2011, Porter et al. 2008). Sequencing coverage of fungal and bacterial communities was estimated using Good's coverage estimator (Good 1953). OTU taxonomic identity was determined using the RDP classifier, and species richness was assessed using Chao1 estimator (Chao 1984, Wang et al. 2007). Following log transformation of OTU relative abundance, taxonomic β-diversity was calculated using the Bray-Curtis metric. To calculate phylogenetic β-diversity, phylogenetic trees were constructed using FastTree 2 (Price et al. 2010), followed by calculation of weighted UniFrac distance between

pairs of old fields and forests (Lozupone et al. 2006). Sequences were uploaded to the Sequence Read Archive under Bioproject PRJNA259629.

Shotgun metagenomic analysis

To gain insight into the functional potential of soil microbial communities to degrade plant detritus, sequences from shotgun metagenomes were assigned to functional genes involved in the decay of cellulose, chitin, galactose-containing oligosaccharides, lignin, pectin, starch and xylan. Twelve libraries were multiplexed and sequenced on four lanes of the HiSeq Illumina instrument, with 150 bp reads. Bacterial sequences were assigned to 20 genes in 7 substrate categories (Table S2.2) using the SEED model within MG-RAST (Meyer et al. 2008). Fungal functional composition was analyzed following creation of 9 gene databases and 6 substrate categories (Table S2.3) created collectively from the Carbohydrate Active Enzyme database, Peroxibase, the Functional Gene Repository, and NCBI reference sequences (Lombard et al. 2013, Fawal et al. 2013, Fish et al. 2013, Tatusova et al. 2014). For each metagenome, abundance of genes involved in the decay of each substrate category (*e.g.*, cellulose, lignin) was calculated following assignation of metagenome sequences to functional gene databases using the Blastn algorithm (Altschup et al. 1990). Bacterial and fungal gene assignment required 60% minimum sequence homology, e-value cut-off value of 1×10^{-5}, minimum alignment 40 bp and were standardized to the number of sequences with predicted functions (*sensu* Fierer et al. 2012). Pairwise Euclidean distances were calculated for input into multivariate analysis. Metagenomes can be publicly accessed via Project ID 5588 (http://metagenomics.anl.gov).

Extracellular enzyme analysis

Extracellular enzyme assays were conducted in 96-well plates, allowing between 8 and 16 technical replicates per sample. To measure activity of β-1,4-glucosidase, cellobiohydrolase,

β-xylosidase and N-acetyl-β-glucosaminidase (NAGase), we used 200 μM methylumbellyferyl-linked substrates (Saiya-Cork et al. 2002). A 25-mM L-dihydroxy-phenylalanine substrate was used to assay phenol oxidase and peroxidase activity; H_2O_2 (0.12%; 25 mL) was included to assay peroxidase activity. One gram of soil mixed and homogenized in 125 ml of 50 mM sodium acetate buffer for 1 minute. Enzyme assays included 100 μL soil-buffer solution, in addition to 50 μL of substrate. Controls included soil plus buffer, buffer plus substrate, and, for MUB substrates, soil and MUB. Cellobiohydrolase, β-xylosidase, β-1,4-glucosidase and N-acetyl-β-glucosaminidase assays were incubated in the dark at 20 °C for 2 h or 30 minutes. Afterwards, 25 μL of 0.2 M NaOH was added to each well to stop the reaction and increase fluorescence. Enzyme activity was measured in a Molecular Devices f-MAX fluorometer set at 365 nm excitation wavelength and 460 nm emission wavelength. Phenol oxidase and peroxidase assays were incubated for 24 h and rates were estimated spectrophotometrically (Saiya-Cork et al. 2002). Enzyme activities were expressed as μmol h^{-1} g^{-1}. Following square-root transformation, pairwise Euclidean distances between sites were calculated for multivariate analysis.

Statistical analysis

Univariate and multivariate statistics were employed to understand relationships between environmental variables, microbial communities, and functional characteristics across old fields and forests. We used regression analysis to understand relationships between time, environmental characteristics, and microbial richness; assumptions of linearity were verified prior to regression analysis. Mantel tests quantified correlations between variation in environmental characteristics, microbial composition, and functional potential. Permutational multivariate analysis of variance (PerMANOVA) tested whether differences in plant functional composition occurred between youngest and oldest abandoned fields. Further, functional and

compositional relationships between sites were visualized using principal coordinate analysis (PCoA). Further, Pearson correlation coefficients were calculated to quantify correlations between individual taxa and principal coordinate axes. Redundancy and distance-based redundancy analysis (RDA, db-RDA) determined the extent to which microbial community composition and functional potential were related to z-transformed environmental variables (Legendre and Anderson 2006). PerMANOVA determined significance of vectors after 9,999 permutations, and forward-stepping selection determined the model significantly describing variation in microbial response variables ($\alpha < 0.05$) with the lowest AIC value. Statistical tests were conducted using the vegan package in R (http://www.R-project.org).

RESULTS

Environmental characteristics

Soil and root analysis confirmed that soil properties across the old-field chronosequence changed through time as a result of plant litter production (Zak et al. 1990, Knops and Tilman 2000). SOM ($r^2 = 0.58$; $P = 0.018$) and soil N ($r^2 = 0.67$; $P = 0.007$) increased with site age; whereas, no relationship was found between old-field age and soil pH ($r^2 = 0.13$; $P = 0.32$). While no linear relationships occurred between the relative dominance of plant functional groups and site age ($P = 0.16 – 0.43$), PerMANOVA revealed that the functional composition of fields comprising the early portion of our chronosequence (16 to 52 yrs) was significantly different from the oldest (61 to 86 yrs), with a higher representation of C3 grasses and forbs as well as fewer C4 grasses in the youngest old fields (Pseudo-$F_{2,9} = 9.05$; $P = 0.024$). Further, lignin:N was positively correlated with C4 relative dominance ($r^2 = 0.74$; $P = 0.003$). Taken together, these results indicate that the increased abundance of C4 grasses across the old-field

chronosequence occurred concomitantly with an increase in root lignin:N, evidence for a progressive change in litter biochemistry over time.

Taxonomic and phylogenetic composition of microbial communities

We used taxonomic and phylogenetic analyses to investigate whether turnover in the soil microbial community paralleled that in the plant community. Analysis of 71,028 non-chimeric fungal sequences resulted in 1,851 unique sequences, with an average sequence length of 385 bp. A total of 1,754 OTUs were identified at 99% 28S sequence similarity. Representing 88% of total sequences, primers preferentially amplified Basidiomycota (Edwards and Zak 2010), with Agaricales comprising the most abundant order. The remaining OTUs were classified as Ascomycota, Blastocladiomycota, Chytridiomycota and Glomeromycota. Good's coverage estimates for 28S sequencing ranged from 0.76 - 0.85 per site, indicating that sites were relatively well sampled, although some rare members were not captured in sequencing effort. Analysis of 71,028 bacterial sequences resulted in 4,968 unique sequences and 3407 bacterial OTUs clustered at 97% similarity, ranging from 440 to 530 bp in length. Despite identical sequencing effort, Good's coverage estimates for bacteria were lower than fungi, ranging from 0.59 - 0.76 per site. With decreasing abundance, bacterial OTUs fell into the phyla of Acidobacteria, Proteobacteria, Actinobacteria, Bacteriodetes, Gemmatimonadetes and Planctomycetes.

Fungal OTU richness estimates, calculated by the Chao1 indicator, ranged from 6,990 to 15,635 OTUs, with highest richness occurring in the 61-yr old field and lowest characterizing the youngest site (16 yrs). Conversely, highest bacterial richness occurred in the youngest field (16 yrs; 10,594 OTUs), whereas the oldest field had the lowest bacterial richness (86 yrs; 8,604 OTUs). Bacterial richness of forest sites ranged between 6,033 and 8,394 OTUs in the UPO and

NH forests, respectively. Fungal OTU richness of the old fields was positively related to time since agricultural abandonment (Figure 2.1; $r^2 = 0.71$; $P = 0.0044$). No relationship was found between bacterial OTU richness and old-field age ($P = 0.13$), but richness was positively related to soil pH across the old fields ($r^2 = 0.53$; $P = 0.027$).

Taxonomic analysis of fungal and bacterial communities revealed changes in composition across the chronosequence, as well as distinct compositional shifts between old fields and late successional ecosystems (Figure S2.1). Of the 10 most abundant fungal families, those unclassified were negatively correlated to site age ($r^2 = 0.48$; $P = 0.037$) and the proportion of sequences assigned to Clavariaceae increased ($r^2 = 0.59$; $P = 0.016$). When comparing old fields to late successional ecosystems, the proportion of sequences classified as Russalaceae (36.3% $\pm$ 3.7% *vs.* 0.90% $\pm$ 0.88%, forest *vs.* old field respectively) and Theleophoraceae (8.40% $\pm$ 1.02% *vs.* 0.27% $\pm$ 0.23%) were significantly higher in forests relative to old-fields. However, due to the large number of unclassified OTUs at the family level (51%), a comprehensive understanding of fungal community dynamics may require phylogenetic analysis. Of the most abundant bacterial families, the proportion of sequences assigned to Acidobacteria Gp1 ($r^2 = 0.44$; $P = 0.053$) and Gemmatimonaceae ($r^2 = 0.44$; $P = 0.052$) negatively correlated to site age, albeit both relationships were marginally significant. Further, Acidobacteria Gp1 and Acidobacteria Gp2 decreased with increasing soil pH ($r^2 = 0.50$ - 0.62; $P < 0.010$) while Chitinophagaceae and Gemmatimonadaceae increased with pH ($r^2 = 0.48$ - 0.53; $P = 0.0070$ - 0.013). Further, the proportion of 16S sequences classified as Acidobacteria Gp2 were higher in forests (6.5% $\pm$ 2.2%) than old fields (2.1% $\pm$ 0.29%); whereas, the relative abundance of Gemmatimonadaceae was lower in forests (2.1% $\pm$ 0.21%) than old fields (4.4% $\pm$ 0.41%).

Phylogenetic analysis of fungal and bacterial communities confirmed taxonomic trends in community turnover observed across the old-field chronosequence and forests. Calculated from weighted UniFrac distance, site comparisons in fungal phylogenetic distance varied from 30 to 78%, and bacterial pairwise differences ranged from 11 to 32%. Visualized in ordination space, phylogenetic differences between fungal communities formed three distinct clusters (Figure 2.2A), whereby forests communities were differentiated from old fields on PCo1. The youngest old fields (16 – 52 yrs) clustered from the oldest old fields (61 – 86 years) along PCo2; this axis was inversely correlated with site age ($P = 0.002$), SOM ($P = 0.010$), and C4 relative dominance ($P = 0.025$). Bacterial communities in forests also differed from the old fields (Figure 2.2B), as demonstrated by separation across PCo1. Interestingly, forest bacterial communities did not cluster tightly as those for forest fungal communities. Bacterial phylogenetic β-diversity between old fields resulted in variation across PCo2, although neither ordination axis varied with site age.

Factors structuring soil microbial β-diversity

To test the hypothesis that changes in the production and biochemistry of plant detritus shaped soil microbial communities, we included SOM, soil pH, root cellulose, root lignin and relative dominance of C3 and C4 grasses in the db-RDA global model to account for variation in phylogenetic β-diversity. Results indicated that SOM composed the best model explaining fungal phylogenetic β-diversity (Pseudo-$F_{1,7} = 2.96$; $P < 0.005$; AIC = 1.48), accounting for 30% of the variation in pairwise UniFrac distance across the old-field chronosequence. Bacteria phylogenetic β-diversity was best modeled by variation in soil pH (Pseudo-$F_{1,7} = 1.94$; $P = 0.035$; AIC = -18.2), accounting for 22% of the variation in UniFrac distance (Figure 2.3A).

Factors structuring microbial functional potential

Microbial functional characteristics, quantified as the relative abundance of functional genes involved in litter decay and soil enzyme activity, were hypothesized to increase with changes in resource availability (*i.e.,* plant detritus production and biochemistry) during secondary succession. The relative abundance of fungal and bacterial genes encoding pectin degradation decreased with site age (r^2 = 0.54 - 0.73; P = 0.004 - 0.028); whereas, no other substrate categories were related to time since agricultural abandonment (P = 0.20 – 0.53). With change in soil pH across old fields, the relative abundance of bacterial genes involved in pectin degradation increased (r^2 = 0.53; P = 0.026) and genes encoding lignin decay declined (r^2 = 0.53; P = 0.025). Total enzyme potential increased across the chronosequence (r^2 = 0.47; P = 0.042), a relationship that appeared to be driven by increasing activity of enzymes involved in decay of cellulose (r^2 = 0.46; P = 0.046) and xylan (r^2 = 0.87; P = 0.0003).

Comparisons between sites were calculated for each functional variable using the Euclidean distance metric, and pairwise dissimilarity of these three variables ranged from 0.6 to 9.5%. PCoA visualizations of microbial functional characteristics across all 12 sites (Figure 2.2C-E) illustrated that forests largely did not serve as functional end-points to old-field succession. Fungal functional gene assemblages clustered in the center of ordination space in Figure 2.2C, whilst old fields scattered across full spectrum of PCo1 and PCo2. PCo1 correlated with enzymes involved in the decay of pectin (r = -0.89; P = 0.0001) and galactose-containing oligosaccharides (r = -0.72; P = 0.0071). PCo2 was correlated to the relative abundance of ligninolytic (r = -0.64; P = 0.025) and xylanolytic genes (r = 0.74; P = 0.0056). Bacterial functional gene assemblages of forest ecosystems were separated along PCo1 of Figure 2.2D, an axis and positively correlated with pectin degrading enzymes (r = 0.75; P = 0.005) and negatively correlated to lignin decay (r = -1; P < 0.0001). Separation of old fields observed PCo2

positively correlated with enzymes metabolizing cellulose (r = 1; $P < 0.0001$) and negatively correlated with chitin, starch and xylan (r = -0.60 – -0.92; $P = 0.0003$ - 0.041). Variation in enzyme potential along both PCos of Figure 2.2E indicated no predictable pattern of enzyme activity in the late-successional forests. PCo1 significantly correlated with an increase in β-1,4-glucosidase, cellobiohydrolase, NAGase and β-1,4-xylosidase (r = 0.60 – 0.95; $P = 0.00001$ - 0.040). NAGase was also positively correlated with PCo2 (r = 0.80; $P = 0.0017$).

Using the same protocol from microbial community analysis, we investigated whether changes in the production and biochemistry of plant detritus also shaped the functional characteristics of the soil microbial community. Results from redundancy analysis (Figure 2.3B) revealed that C4 grass relative dominance was the best model to explain variation in fungal functional gene composition (Pseudo-$F_{1,7} = 3.65$; $P = 0.030$; AIC = 21.0), accounting for 26% of total variation. The relative dominance of C3 grasses also significantly accounted for variation in fungal functional composition (Pseudo-$F_{1,7} = 2.38$; $P = 0.026$; AIC = 20.99). Considering bacterial functional composition, soil pH was marginally significant, accounting for 36% of variation across the old-field chronosequence (Pseudo-$F_{1,7} = 4.10$; $P = 0.083$; AIC = -31.8). SOM was the best model explaining enzyme potential (Pseudo-$F_{1,7} = 13.7$; $P = 0.005$; AIC = 10.4), capturing 66% of the variation in enzyme activity across old fields (Figure 2.3A), but C4 relative dominance was also a significant predictor (Pseudo-$F_{1,7} = 6.95$; $P = 0.017$; AIC = 13.9).

Link between community composition and functional potential

Mantel correlations tested the hypothesis that fungal and bacterial β-diversity was linked to variation in microbial functional potential. Analysis revealed fungal phylogenetic dissimilarity was correlated to extracellular enzyme potential (R = 0.44; $P = 0.019$), and marginally related to variation in functional gene composition (R = 0.33; $P = 0.067$). We found a significant

correlation between bacterial UniFrac distance and variation in bacterial functional gene composition (R = 0.60; $P = 0.002$), indicating bacterial functional traits were linked to bacterial phylogenetic relatedness. However, we observed no relationship between bacterial UniFrac distance and extracellular enzyme potential ($P = 0.36$).

DISCUSSION

Our observations support the hypothesis that changes in fungal communities parallel those in plant communities across the old-field chronosequence; whereas, edaphic properties may serve as a more important filter for soil bacteria during secondary succession. Evidence consistent with these ideas comes from the fact that changes in SOM content across the old-field chronosequence accounted for increased fungal OTU richness and fungal phylogenetic β-diversity. Secondly, observed changes in fungal gene assemblages with C4 grass relative dominance indicate that changes in litter biochemistry across the old-field chronosequence may select for fungi according to their physiological capacity to metabolize organic substrates in plant detritus. While soil bacteria were not sensitive to changes in plant communities, soil pH accounted for differences in bacterial richness, phylogenetic β-diversity and the composition of bacterial genes mediating litter decay. Lastly, concomitant changes in fungal functional gene assemblages and enzyme activity provide evidence that changes to the soil microbial community have direct consequences to the functional potential of the soil community to degrade plant detritus. Inasmuch, our observations partially support our overall hypothesis that saprotrophic microbial communities in soil are structured by changes in the biochemical composition of detritus as plant composition changes during secondary succession.

Resource availability shapes fungal communities and their capacity to metabolize plant detritus

During old-field succession, changes in resource availability appear to structure the community composition of soil fungi. Increasing fungal OTU richness paralleled SOM accumulation (Figure 2.1), suggesting that a greater number of fungal taxa were able to meet minimum resource requirements as resource availability increased over successional time (Tilman 1980, Waldrop et al. 2006). Because the late-successional forests had lower fungal OTU richness relative to the oldest fields, a unimodal relationship may exist between resource availability and fungal richness, wherein competitively superior taxa dominate at higher resource levels (Kassen et al. 2000, Waldrop et al. 2006). Secondly, variation in SOM accounted for fungal phylogenetic turnover from youngest to oldest fields (Figure 2.3), indicating that fungal community membership successively shifted with greater resource availability (Tscherko et al. 2004). Consistent with our observations, the correlation between fungal β-diversity and increasing soil C and N across a glacial chronosequence (Zumsteg et al. 2012) points to resource availability as an ecological driver of fungal succession. Further, the idea that saprotrophic soil fungi possess ecological trade-offs that influence their competitiveness for growth-limiting substrates is consistent with our observations. However, evaluating this expectation requires detailed understanding of the physiological capabilities of the fungal taxa in our study as well as species-to-species interactions among them (Boddy 2000).

During secondary succession, changes in the abundance and biochemical composition of plant detritus appear to shape the functional potential of the fungal community to degrade organic substrates. For example, overall enzyme potential increased with SOM, suggesting that the accumulation of plant detritus through time supported a more active microbial community (Tscherko et al. 2004). This may be the result of an increase in fungal OTU richness or an increase in microbial biomass (Zak et al. 1990, Waldrop et al. 2006). Changes in plant litter

biochemistry also appeared to structure fungal functional characteristics, as the relative dominance of C4 grasses predicted temporal changes in the composition of fungal genes mediating litter decay. This link between plant functional groups and litter biochemistry is supported by the positive correlation between C4 grasses and root lignin content, as well as increasing organic matter C:N (Knops and Tilman 2000, Quested et al. 2007).

Due to increased dominance of C4 grasses across the old-field chronosequence, a greater abundance of lignified plant detritus provides a plausible force driving competitive displacement of fungal taxa. Rapid turnover of fungi during the decay of an individual leaf indicates that composition changes as the biochemical constituents of detritus are differentially metabolized (Voříšková and Baldrian 2013). Further, lignin content and biochemical composition are major factors influencing fungal succession during the decay of leaves (Osono 2007). Taken together, our results suggest that plants drive compositional changes in soil microbial communities during secondary succession via the continual input of detritus, as well as differences in litter biochemistry among plant species (van der Wal et al. 2013, Cutler et al. 2014).

Soil pH structures bacterial communities and their genetic capacity to degrade organic material

Unlike fungi, bacterial OTU richness and phylogenetic β-diversity appear to be influenced by changes in soil pH across the old-field chronosequence, indicating that edaphic factors are the primary agent structuring bacteria communities (Fierer and Jackson 2006, Rousk et al. 2010). Increasing soil pH resulted in increased OTU richness, results consistent with the idea that deviations in soil pH may introduce stress on single-celled organisms and limit the survival of taxa outside of their pH optimum (Kowalchuk et al. 2002, Fierer and Jackson 2006). Further, it is plausible that this stress could alter competitive outcomes and drive community turnover. For example, changes in soil pH altered the abundance of Chitinophagaceae,

Gemmatimonadaceae and families assigned to Acidobacteria (Lauber et al. 2009, Bajerski and Wagner 2013). Providing support to this assertion, studies report community turnover is structured by changes in geologic substrate age and soil pH, but are not sensitive to changes in plants across a successional sequence (Kuramae et al. 2011, Cutler et al. 2014). Yet, others find that bacterial communities mirror changes in plant communities during succession (Mitchell et al. 2010). Because variation in soil pH across the old-field chronosequence did not follow time since agricultural abandonment, our findings contrast observations of predictable changes in bacterial communities with successional time (Nemergut et al. 2007), and indicate that edaphic factors may alter the trajectory of bacterial succession.

Soil pH appears to also shape bacterial functional gene assemblages, indicating that consistent filters structure changes in bacteria community composition and genetic potential to degrade organic substrates. Redundancy analysis, in addition to the altered abundance of genes involved in cellulose, pectin and lignin decay, provide evidence that soil pH has direct consequences to the bacterially mediated decay of organic substrates. It appears that bacteria are selected on account of pH tolerance and corresponding changes in functional assemblages are the consequence of phylogenetically conserved traits in a changing bacterial community; however, further experimental manipulations are necessary to test this hypothesized mechanism.

Microbial community composition and functional potential: are there links?

Wide variation in phylogenetic dissimilarity between communities, yet a relatively narrow range of variation in functional gene composition and enzyme potential, indicates the presence of a core set of metabolic capacities within fungal and bacterial communities (Burke et al. 2011, Talbot et al. 2014) and further suggests that soil microbial communities may exhibit a degree of functional overlap across the old-field chronosequence. Yet, predictable shifts in the

functional traits of the soil microbial community with phylogenetic community suggests that change in composition has consequences for the genetic and enzymatic potential for microbial communities to degrade organic substrates. Significant correlations between variation in enzyme potential and fungal β-diversity support the idea that changes in fungal composition across the chronosequence the potential of the microbial community to decompose organic material. In contrast, the lack of correspondence between bacterial β-diversity and enzyme potential indicates that bacteria comprise a relatively small fraction of the saprotrophic soil community in our chronosequence, because sandy, dry, and acidic soils favor fungi (Paul and Clark 1990, Zak et al. 2003). The marginally significant correlation between fungal function and composition suggests other functional traits may also be important in determining access to resources, such as growth efficiency, dispersal ability, and combative mechanisms of competition (Boddy 2000, Bissett et al. 2010). Quantifying a wider range of traits of bacterial and fungal communities is necessary to further understand the primary functional traits that shape soil microbial communities.

Forests as potential endpoints to the succession of microbial communities

Distinct differences between fungal and bacterial community composition beneath forests and old fields provides evidence that communities change substantially from oldest old fields (86 yrs) to late-successional ecosystems (Figure 2.2). The separation of fungal communities in forest ecosystems from old fields was driven by an increase of Russalaceae and Theleophoraceae, and demonstrated a well-established trend towards increasing ectomycorrhizal associations with forest development (Kranabetter et al. 2005, Twieg et al. 2007). While we expect to see a gradual increase in ectomycorrhizal associations as grasslands give way to forests, additional sampling of sites of early forest development are necessary to test whether compositional and functional

characteristics of soil microbial communities converge towards forests with increasing age. The fungal gene assemblages beneath forests were largely nested within functional variation across the old-field chronosequence, providing further evidence that fungal communities have overlapping functional capabilities to degrade organic substrates. Similarly, forest bacterial communities were also distinct in the late-successional forests, marked by an increase in Acidobacteria and lower abundance of Gemmatimonadaceae relative to old fields. However, blurred distinctions between old field and forest bacterial communities in ordination spaced may reinforce evidence that bacterial are less sensitive to plant species differences (Cutler et al. 2014).

Conclusion

Here, we have demonstrated that changes in fungal communities and functional potential during secondary succession appear driven by variation in resource availability and litter biochemistry; whereas, bacterial communities respond to variation in edaphic properties. Distinct differences between old-field and forest microbial communities further indicated that substantial turnover occurs during microbial succession, as old fields give way to forests in this landscape. We provide evidence that changes in fungal and bacterial composition communities have consequences to the genetic and metabolic potential of soil microbial community to harvest energy from organic substrates. Yet, smaller differences in the functional potential of saprotrophic bacterial and fungal communities relative to community turnover indicate some degree of overlapping functional abilities within soil communities, pertaining to the physiological capacity to metabolize plant detritus. Investigation of the relationship between genetic potential and gene regulation through meta-transcriptomics and meta-proteomics may be required to capture the mechanism linking microbial community composition and function.

Nevertheless, our results indicate that changes in plant communities during secondary succession cause concomitant changes in the composition of fungal communities and has direct consequences to the decomposition of plant litter.

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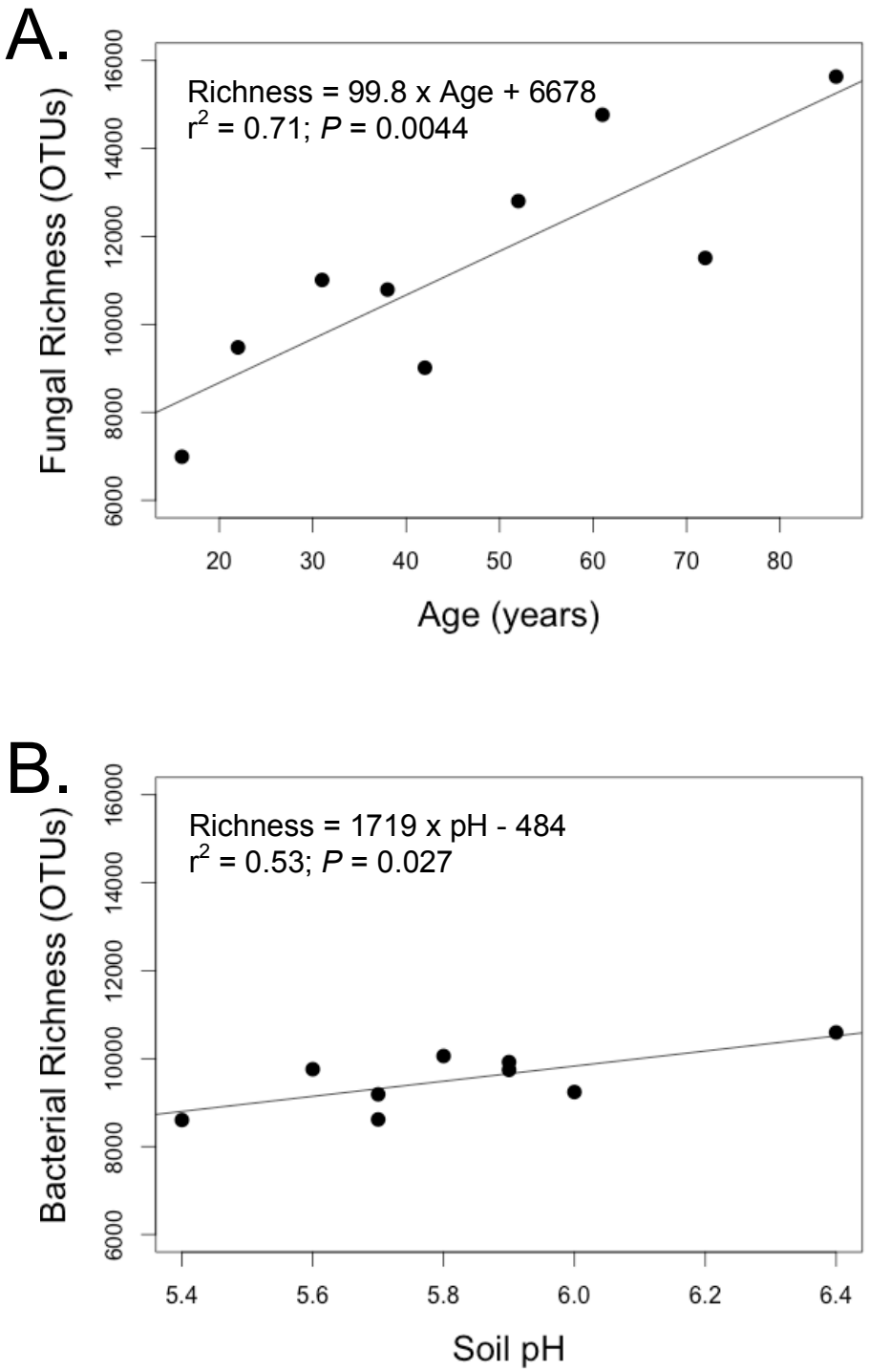

Figure 2.1. Linear regression analysis of fungal (A) and bacterial (B) OTU richness. Estimated fungal OTU richness significantly increased with time since agricultural abandonment; whereas, bacterial OTU richness significantly increased with soil pH. OTUs were clustered at 99% (28S) and 97% (16S) sequence similarity. OTU richness was calculated by the Chao1 estimator.

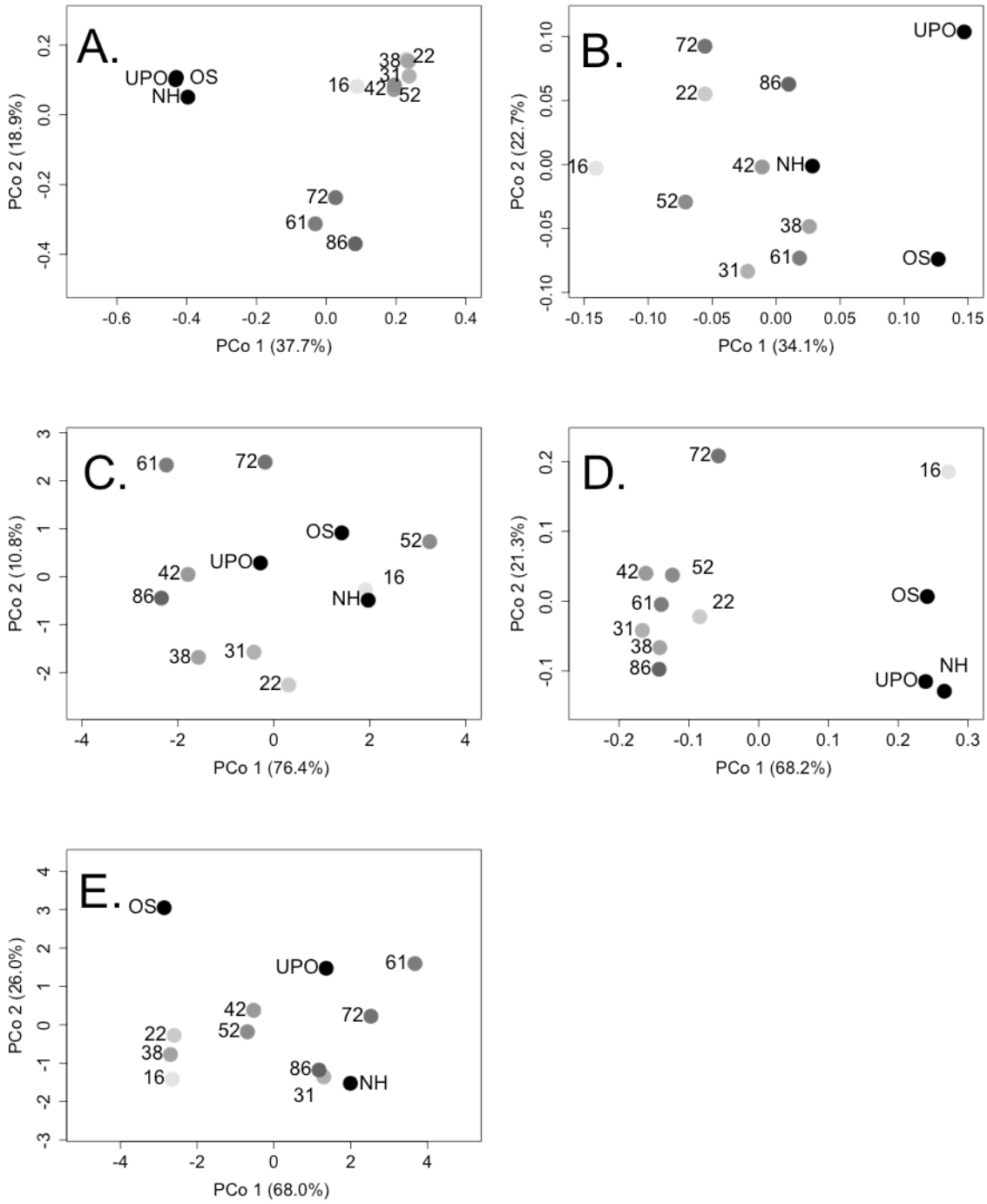

Figure 2.2. Principal components analysis of fungal phylogenetic composition (A), bacterial phylogenetic composition (B), fungal functional gene composition (C), bacterial functional gene composition (D) and extracellular enzyme potential (E). Phylogenetic distances were calculated by the weighted UniFrac distance metric. The Euclidean distance metric was used to calculate pairwise site differences in functional characteristics. Numbers indicate field age and shading represents relative age with young fields in light gray, older fields in dark gray. Three forested sites (NH = Northern Hardwoods, OS = Oak Savanna and UPO = Upland Pin Oak) are in black.

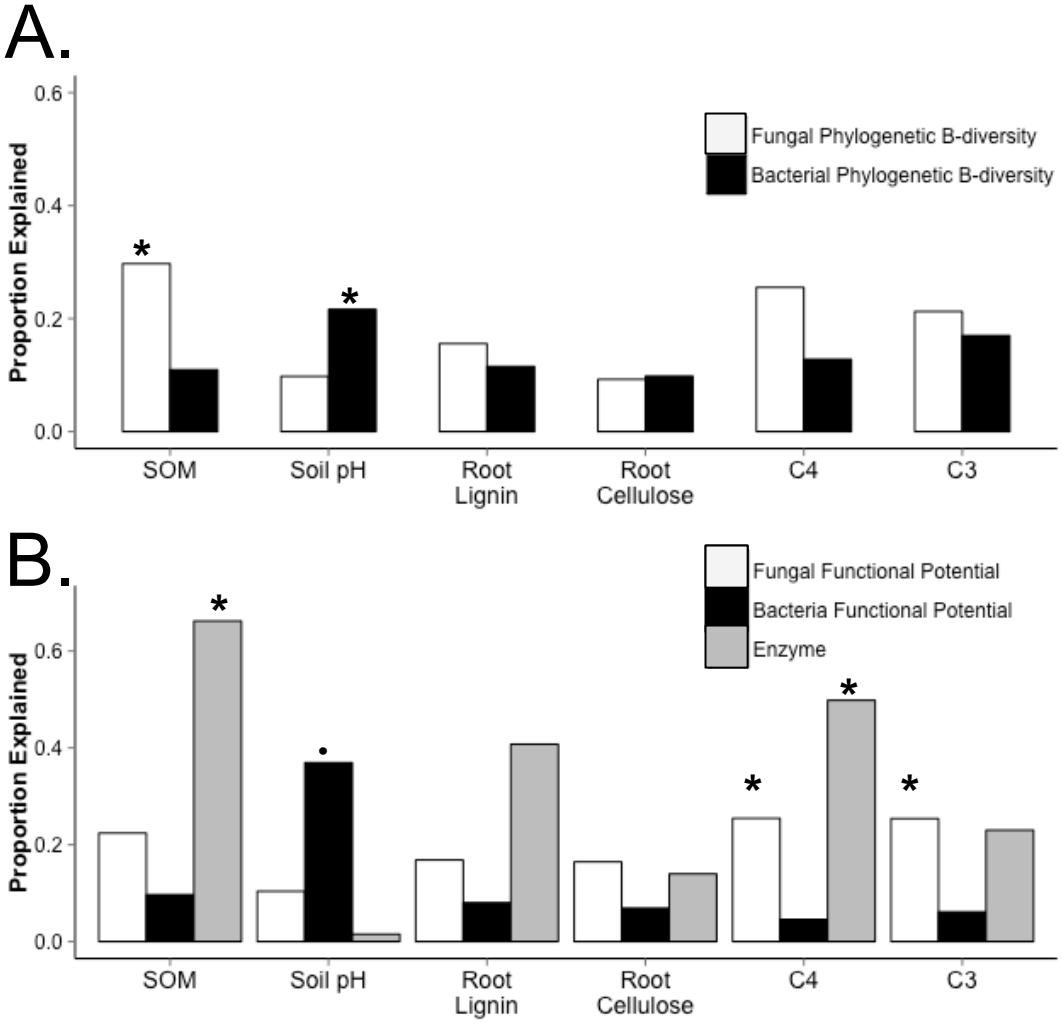

Figure 2.3. Proportion of variance explained in microbial phylogenetic β-diversity (A) and functional potential (B) by individual variables included in redundancy analysis across the old-field chronosequence. Soil organic matter (SOM) significantly accounted for in fungal phylogenetic β-diversity (Pseudo-$F_{1,7} = 2.96$; $P < 0.005$) and variation in enzyme activity ($F_{1,7} = 13.7$, $P = 0.005$). Variation in soil pH significantly modeled bacteria phylogenetic β-diversity (Pseudo-$F_{1,7} = 1.94$; $P = 0.035$; AIC = -18.2) and was marginally significant in accounting for variation in bacterial functional potential (Pseudo-$F_{1,7} = 4.10$, $P = 0.083$). Variation in fungal function potential was significantly accounted for by C4 (Pseudo-$F_{1,7} = 3.65$, $P = 0.030$) and C3 grass relative dominance (Pseudo-$F_{1,7} = 2.38$, $P = 0.026$). C4 relative dominance also significantly accounted for variation in enzyme potential (Pseudo-$F_{1,7} = 6.95$, $P = 0.017$). An * denotes significance at $\alpha < 0.05$; whereas · represents significance at $\alpha < 0.10$.

SUPPLEMENTARY TABLES

Table S2.1. Soil, root and plant functional characteristics collected for each of the nine sites in the old-field chronosequence and three forests. NH = northern hardwoods, OS = oak savanna, UPO = upland pin oak. Plant biomass was not collected for forest communities.

Site Age (y)	**16**	**22**	**31**	**38**	**42**	**52**	**61**	**72**	**86**	**NH**	**OS**	**UPO**
Soil Characteristics												
pH	6.4	5.8	5.7	5.4	5.9	5.9	6	5.6	5.7	5.2	5.8	5
SOM (mg/g)	11	11	14	13	11	14	24	18	19	40	33	27
Total N (μg/g)	859	821	1063	946	868	1139	1274	1132	1220	1902	1942	1262
Root Characteristics												
Lignin (mg/g)	66	7.0	72	35	131	87	161	74	101	289	333	318
Cellulose (mg/g)	692	623	657	837	454	314	385	719	624	433	272	364
Hemicellulose (mg/g)	74	147	115	57	185	363	254	100	64	36	94	23
Lignin:N	10.7	11.4	14.0	10.5	14.8	9.0	27.3	14.7	12.7	38.7	29.1	24.6
Plant Characteristics (% Relative Dominance)												
C3 grass	74.0	38.1	83.5	46.0	42.8	90.0	7.4	26.0	31.8			
C4 grass	14.8	37.0	15.2	14.1	34.7	0.1	86.0	59.0	43.7			
Forb	5.7	18.2	0.1	14.0	22.1	6.9	2.1	0.1	4.2			
Legume	5.5	2.2	1.1	25.5	0.3	0.0	0.0	0.0	0.0			

Table S2.2. Summary of functional genes used in metagenomic analysis of bacterial functional potential to degrade plant and microbial litter.

Enzyme (Gene) Name	EC Number	Substrate Category
Beta-glucosidase	3.2.1.21	Cellulose
Endocellulase	3.2.1.4	Cellulose
Alpha-N-acetylglucosaminidase	3.2.1.50	Chitin
Chitin deacetylase	3.5.1.41	Chitin
Endochitinase	3.2.1.14	Chitin
β-N-acetylhexosaminidase	3.2.1.52	Chitin
Alpha galactosidase	3.2.1.22	Galactose-containing Oligosaccharides
Laccase	1.10.3.2	Lignin
Endopolygalacturonase	3.2.1.15	Pectin
Exopolygalacturonase / galacturan 1,4-α-galacturonidase	3.2.1.67	Pectin
Pectate lyase	4.2.2.2	Pectin
Pectin lyase	4.2.2.10	Pectin
Pectinesterase	3.1.1.11	Pectin
Alpha-amylase	3.2.1.1	Starch
Alpha-glucosidase	3.2.1.20	Starch
Glucan 1,4-alpha-glucosidase	3.2.1.3	Starch
Alpha-L-arabinofuranosidase	3.2.1.55	Xylan
Beta-xylosidase	3.2.1.37	Xylan
Endo-1,4-ß-xylanases	3.2.1.8	Xylan

SUPPLEMENTARY FIGURES

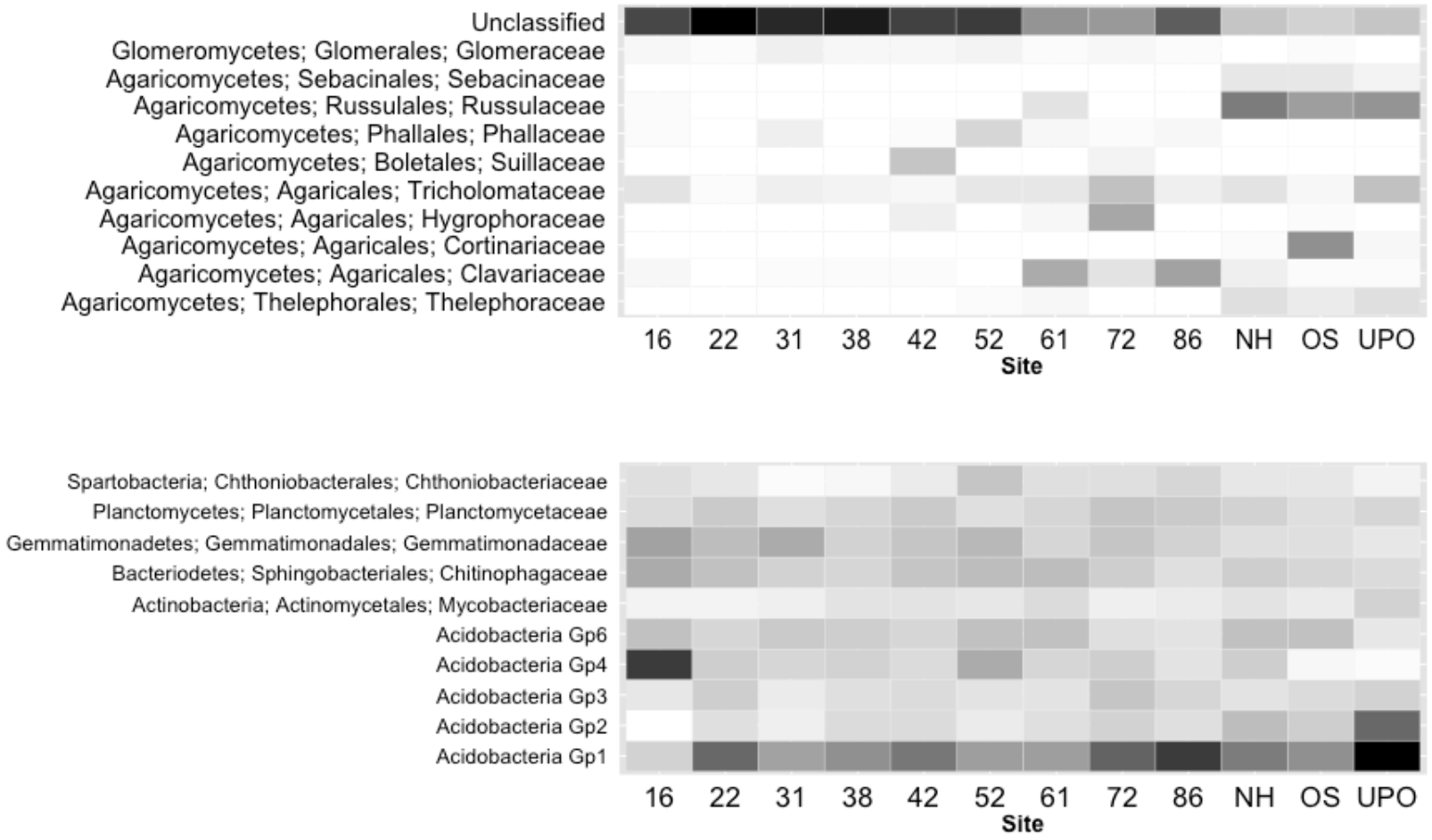

Figure S2.1. Changes in the relative abundance of the ten most abundant fungal (A) and bacterial (B) families across the old-field chronosequence and late successional ecosystems. Numbers represent old fields by time (yrs) since agricultural abandonment. Letters represent the three forested sites (NH = Northern Hardwoods, OS = Oak Savanna and UPO = Upland Pin Oak). Light shades represent low relative abundance of microbial taxa and darker shades of gray to black higher relative abundance.

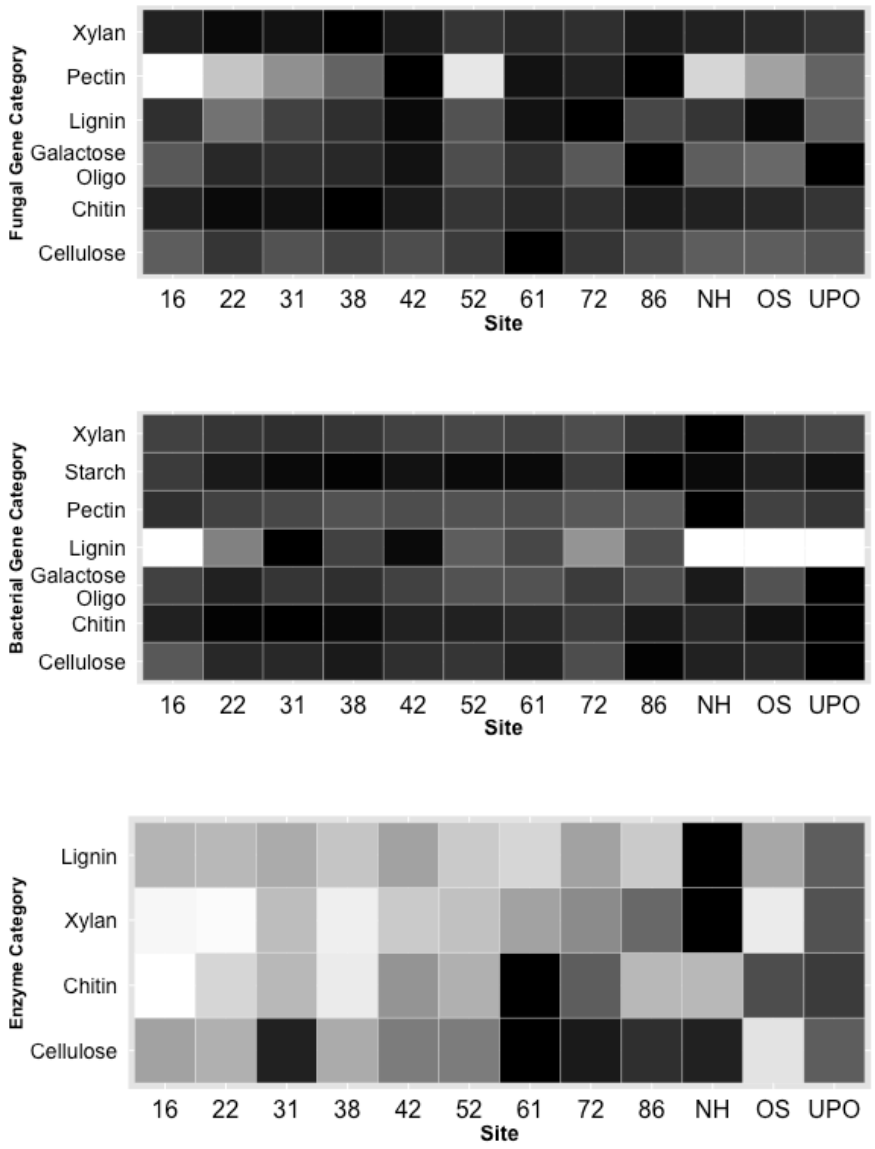

Figure S2.2. Changes in the functional potential of soil microbial community to degrade components of dead organic material across the old-field chronosequence and late successional ecosystems according to three different measures, relative abundance of fungal genes (A), relative abundance of bacterial genes (B), and enzyme activity (C). Numbers represent old fields by time (yrs) since agricultural abandonment. Letters represent the forested sites (NH = Northern Hardwoods, OS = Oak Savanna and UPO = Upland Pin Oak). Light shades represent low relative abundance of microbial taxa and darker shades of gray represent higher relative abundance. Relative abundance of substrate categories were expressed as proportion of sequences with predicted functions. For visualization in heatmap, each substrate category was standardized relative to its maximum observed value to downweight substrate categories with magnitudinally higher relative abundances. Enzyme activities were expressed as μmol h^{-1} g^{-1} and square root transformed to downweight abundant gene and enzyme categories.

CHAPTER III

Dispersal limitation structures fungal community assembly in a long-term glacial chronosequence

ABSTRACT

Microbial communities in soil mediate biogeochemical processes; however, understanding forces shaping their composition and function remains a gap in our ecological knowledge. We investigated phylogenetic turnover and functional gene composition of saprotrophic fungi along a 4000-year glacial chronosequence. A direct relationship between β-diversity and geographic distance, a proxy for time since deglaciation, indicated that dispersal limitation shapes saprotrophic fungal communities. Further, we infer dispersal limitation may also influence fungal functional properties, as metabolic potential and functional richness increased with site age. Despite attempts to minimize environmental variation, a direct relationship between β-diversity and biogeochemical differences across sites indicated environmental filtering further shapes fungal community composition. However, environmental filtering was overshadowed by the effect of dispersal limitation when tested by multiple regression. Fungal β-diversity and composition of functional genes involved in plant litter decay were unrelated, suggesting that functional traits are not phylogenetically conserved across this chronosequence. Our study suggests that dispersal limitation operates in structuring present-day fungal community composition and functional potential. Further, we demonstrate the need to

integrate functional and phylogenetic approaches to more accurately portray microbial communities and their functional capacities.

INTRODUCTION

As primary decomposers in terrestrial systems, understanding the ecological forces and timescales shaping saprotrophic microbial communities remains an important gap in our ecological knowledge. Identifying the rules by which soil microbial communities assemble has been hampered by their complexity, as well as prior conceptual and methodological limitations (Prosser et al. 2007). Due to their small size and rapid generation times, microbial communities were broadly believed to assemble swiftly in the absence of geographic or biological barriers to dispersal (Finlay, 2002). This perception resulted in the widespread hypothesis that, with regards to microorganisms, "everything is everywhere – the environment selects" (Baas Becking 1934). Further, the remarkable diversity of soil microorganisms contributed to the belief that most are functionally equivalent.

In addition to environmental filtering, mounting evidence indicates priority effects and dispersal limitation may shape microbial community assembly (Fukami et al. 2010, Martiny et al. 2011, Hanson et al. 2012). Over time, ecological drift differentiates communities that are dispersal limited through stochastic local species extinctions (Vellend, 2010). In contrast, effective dispersal can lessen the strength of ecological drift as propagules from regional species pools enter a local community. Dispersal can also influence the sequence of colonist arrival, potentially shaping the trajectory of community assembly due to priority effects (Wilbur and Alford, 1985, Dickie et al. 2012). Environmental factors can act as "filters", modifying community composition by constraining the occurrence of species or traits that make organisms poor competitors under specific conditions (Keddy 1992). At a global scale, ecological drift may be a significant force, because physical and ecological barriers limit gene flow among microbial communities (Papke and Ward 2004). Physical and biotic factors appear to govern community

composition at local landscapes (Edwards and Zak 2010, Stegen et al. 2012). However, the interplay between historical processes and environmental filtering, particularly at intermediate spatial and temporal scales, remains unresolved (Martiny et al. 2011).

Despite recent developments, the relationship between microbial community composition and belowground processes is poorly understood (Van der Heijden et al. 2008). Due to physiological differences in the metabolism of organic substrates, rates of resource use and soil C storage may wary with shifts in microbial composition (Zhang et al. 2007, McGuire et al. 2010). Phylogenetic clustering of functional traits (Martiny et al. 2012, Treseder et al. 2011) provides a potential mechanism linking the composition of microbial communities to biogeochemical processes in soil. In contrast, others have demonstrated that ecosystem processes are robust to community differences, due to an overlap in metabolic activity of organisms with varying evolutionary histories (Nielsen et al. 2011). Key to addressing this controversy is exploring the relationship between genetic identity and functional trait information (Hättenschwiler et al. 2011). With advances in molecular technologies, microbial ecologists have begun to characterize functional characteristics based on the presence of genes encoding enzymes that mediate key biogeochemical processes (He et al. 2007). Combining the study of microbial biogeography with functional trait analyses holds promise to increasing our mechanistic understanding of the environmental drivers of ecosystem-level processes, as well as the ecological forces structuring soil microbial communities.

Litter-decay fungi serve as a model system to investigate functional biogeography of microorganisms involved in plant litter decomposition. Saprotrophic fungi mediate the decay of lignin, a major component of terrestrial plant litter (De Boer et al. 2005, Baldrian, 2006); moreover, they have the potential for long-distance dispersal and establishment as a result of

spore production (Hallenberg and Kuffer, 2001). While recent studies have focused on the biogeography of plant-associated fungi (e.g., Kivlin et al. 2011, Tedersoo et al. 2012), few have considered community assembly dynamics of free-living saprotrophic fungi (Green et al. 2004, Feinstein and Blackwood 2012). Despite evidence for long-distance wind dispersal of arctic ectomycorrhizal fungi (Bjorbækmo et al. 2010, Timling and Taylor 2012), temperate fungal communities may show strong spatial structure (Edman et al. 2004, Peay et al. 2010). This observation suggests that historical processes, such as community assembly history and dispersal limitation, may have a lasting effect on saprotrophic fungal community composition.

To investigate the ecological mechanisms structuring fungal community composition and function, we quantified phylogenetic β-diversity and functional gene composition across a glacial chronosequence. Glacial retreat presents a unique opportunity to study the factors that structure composition and ecosystem properties through the formation of sites with varying ages yet similar attributes (e.g., Ohtonen et al. 1999). Retreat of the Wisconsin glacier *ca.* 14,000 years ago occurred in a south to north direction across the Upper Great Lakes Region of North America. Distance between sites across this region, serving as a proxy for time since deglaciation, enabled us to investigate the impact of temporal gradients on the assembly of present-day fungal communities. Four northern hardwoods sites were selected along this chronosequence for similar edaphic and floristic characteristics to minimize environmental heterogeneity (Burton et al. 1991). Long-term monitoring of sites enabled us to quantify confounding variation along the temporal gradient as well as investigate the impact of subtle environmental gradients on fungal community composition.

If dispersal limitation is at work at a regional scale, then a positive relationship should exist between fungal community dissimilarity and time since community assembly (*i.e.,* distance

between sites), independent of confounding environmental variation. Conversely, if environmental filtering is at work, then differences in fungal community composition should be related to environmental changes across sites. Furthermore, if community composition and function are linked, then communities that are phylogenetically similar will share a high degree of functional similarity. To test these hypotheses, we analyzed fungal community composition by constructing clone libraries from forest floor DNA and profiled community functional potential using a functional gene microarray (He et al. 2007). Here, we provide evidence suggesting that dispersal limitation operates over the timeframe of centuries to millennia to shape soil fungal communities.

METHODS

Study sites

Forest floor samples were collected in October 2009 from four sugar maple (*Acer saccharum* Marsh.) dominated northern hardwood sites. Study sites span 400 km south to north along Lower and Upper Michigan USA. Locations were selected from a population of 31 candidate sites based on floristic and edaphic similarity (Burton et al. 1991). Soils at these sites are well-drained sandy typic Haplothords of the Kalkaska series. For additional site information, see Table 3.1 and S3.1. At each site, forest floor samples were collected from three 30-m x 30-m replicate plots. Within each plot, 10 random 10-cm x 10-cm forest floor samples were collected and composited after manually removing the Oi horizon. In each plot, Oe and Oa horizons were homogenized by hand with sterilized scissors and a 10-g subsample was taken for laboratory analysis. A total of 12 samples were flash frozen in N_2 liquid and immediately transported to the University of Michigan and stored at -80 °C.

DNA extraction, amplification and molecular cloning

Genomic DNA was extracted from 2.5 g of forest floor material using PowerMax TM Soil Extraction Kits (Mo Bio Laboratories, Solana Beach, CA). Fungal rDNA was selectively amplified using primers 58AF2 (Martin and Rygiewicz, 2005) and LR3 (Hopple and Vilgalys, 1994) targeting the ITS-2 region and large ribosomal segment (28S gene). Duplicate PCR reactions for each composite forest floor sample contained 1 µL of DNA, 0.4 µL of 10 µM forward primer 58AF2, 0.4 µL of 10 µM reverse primer LR3, 2.5 µL dNTPs (2 µM), 2.5 µL 10X PCR buffer (1.5 mM $MgCl_2$; Roche, Hamburg, Germany), 1 µL BSA, 0.5 µL high-fidelity *Taq* polymerase (Roche, Hamburg, Germany), and 16.7µL molecular grade water. Duplicate PCR reactions ensured great enough mass of the targeted region for downstream analysis. After an initial denaturation step of 1 min at 95 °C, cycles of denaturing at 95 °C for 30 s, annealing at 50 °C for 30 s and elongation at 72 °C for 60 s, followed by a final extension step of 72 °C for 15 min, was carried out using Eppendorf PCR cyclers (Hamburg, Germany). Duplicate PCR products were combined and purified (UltraClean PCR Clean-up, Mo Bio), followed by cloning into pCR 2.1-TOPO using the TOPO TA Cloning kit (Invitrogen, Grand Island, NY). Vectors were transformed into TOP10 competent cells. One hundred and ninety two clones were selected from each plot and grown overnight in 96 well plates at 37 ° C in Luria-Bertani broth containing 10% glycerol, 0.025 g L^{-1} ampicilin, 0.0125 g L^{-1} kanamycin. Libraries were submitted for Sanger sequencing at SeqWright (Houston, TX).

Phylogenetic analyses

Sequence histograms were edited and contiguous sequences were constructed using Geneious 5.4.2 (Kearse et al. 2012) for the 12 clone libraries. Clone and reference sequences were aligned with MAFFT in Geneious (Katoh et al. 2002) and manually edited for gaps and

hyper-variable regions. Closest reference sequences were identified using an NCBI BLAST search (Altschup et al. 1990) and incorporated into the alignment. Suspected chimeric sequences were manually identified due to extreme variation in length, non-continuous sequences or grouping with non-fungal reference sequences on preliminary neighbor-joining trees constructed in Geneious. Similarity matrices were generated based on alignments of the homologous 28S region, followed by grouping operational taxonomic units (OTUs) based on 99% sequence similarity using the average neighbor algorithm in Mothur (Schloss et al. 2009, Edwards and Zak, 2010). Sequences representing each OTU have been deposited in GenBank (KC588534-KC588833). OTUs were aligned with basidiomycete and ascomycete reference sequences and a Glomeromycete outgroup (see Table S3.4) using MAFFT in Geneious. A maximum likelihood tree was constructed using PhyML in MEGA 5.0 with GTR selected as the best substitution model using jModeltest (Posada and Crandall 1998, Tamura et al. 2011). This tree was used as input for community analyses based on branch length (see Figure S3.3).

Community composition and structure

Phylogenetic composition within and between communities was quantified using Phylocom version 4.2 (Webb et al. 2008). Phylogenetic diversity (PD) is defined as the minimum branch length spanning any given set of species on a phylogenetic tree. Nearest-taxon-index (NTI) quantified the standard deviation of phylogenetic distance between closest relatives in a community when compared to the phylogenetic distance of closest relatives in a community randomly assembled from the tips of the phylogeny (999 randomizations). Net Relatedness Index (NRI) is calculated as a tree-wide measure of phylogenetic clustering. NTI and NRI scores significantly greater than zero suggests that communities are more closely related than

expected by chance, while negative scores suggest that communities are more distantly related than expected by chance (Webb et al. 2002).

Investigating phylogenetic distance between pairs of communities provided information about β-diversity across distance and environmental gradients. D_p represents an abundance-weighted measure of the pairwise differences in branch length of OTUs in a community relative to another. A higher D_p for an assemblage is indicative of higher phylogenetic diversity relative to the total taxa pool and therefore more phylogenetically distant pairs. βNTI, the between-community analog to NTI, is the deviation of the abundance-weighted phylogenetic distance amongst closest relatives between two communities ($\beta MNTD_{obs}$) as compared to the average phylogenetic distance when both communities are randomized across the phylogenetic tree (mean $\beta MNTD_{null}$). A null distribution was found by shuffling species labels across the phylogeny, the "phylogeny shuffle" null model in Phylocom, and recalculating βMNTD 999 times. Community turnover increases as βNTI departs from the mean of this distribution, providing support for the role of abiotic or biotic forces in structuring β-diversity (Stegen et al. 2012). Because D_p accounts for evolutionary relationships among taxa in both recent and evolutionary time whereas βNTI quantifies distance only between nearest relatives, we believe that these approaches provided a more complete understanding of community differences.

GeoChip 4.0 processing and analyses

Analysis of the metabolic potential of the saprotrophic fungal community was conducted using the GeoChip 4.0 (He et al. 2007). One μg of genomic DNA from each replicate sample was purified by the Genomic DNA Clean & Concentrator kit (Zymo Research, Irvine, CA) and labeled with fluorescent dye Cy-3 using random primers (Wu et al. 2006). The labeled gDNA was dried and rehydrated with 2.7 μl of sample tracking control, followed by incubation at 50 °C

for 5 min. This DNA solution was then mixed with 7.3 µl of hybridization buffer containing the universal standard DNA labeled with Cy-5 dye; it was denatured at 95 °C for 5 min and maintained at 42°C until loaded onto GeoChip arrays (NimbleGen, Madison, WI). The hybridization was performed on a Hybridization Station (MAUI, Roche, CA) at 42 °C for 16 h with agitation. After washings, the arrays were scanned using a MS 200 Microarray Scanner (NimbleGen).

To investigate functional potential of the fungal community to degrade plant and fungal litter, we selected a subset of probes on GeoChip 4.0. Six gene categories were identified from the 'Carbon cycling' genes based on the metabolism of specific substrates, including amylases, cellulases, chitinases, hemicellulases, ligninases and proteases; only fungal functional genes were analyzed. Across all gene categories, 31 functional genes and a total of 1398 probes were used in analysis of functional potential (see Table S3.3). Functional richness, the number of targeted gene variants encoding the same function (*i.e.,* lignin depolymerization), represented the number of targeted probes within a gene category registering a fluorescent signal greater than zero. Overall metabolic potential was calculated as the sum of corrected fluorescent signal intensity of the selected 31 functional genes. Functional composition was calculated as the relative abundance of functional genes within each gene category. Varying proportions of functional genes within each gene category may suggest varying physiological abilities of the fungal community to metabolize plant and fungal detritus. Fluorescent signal intensity (SI) and richness calculations were corrected by the number of probes per gene, and genes per gene category; therefore, individual genes and gene categories were not weighted by an uneven abundance of probes or functional genes on the microarray (Xie et al. 2011).

Differences in total metabolic potential and functional richness across the chronosequence were explored using ANOVA in the software package R 2.11 (http://www.r-project.org/). Post hoc analyses were conducted using Tukey's HSD test. All assumptions of linearity were verified prior to analysis by Shapiro-Wilks tests and inspection of residual plots. To investigate the factors contributing to site differences in functional richness and composition of the six functional gene categories involved in litter decay, two distance matrices were created. Pairwise comparisons of functional richness were calculated by the Sørenson dissimilarity metric. The Bray-Curtis dissimilarity metric was used to generate differences in functional composition across sites.

Environmental analyses

Environmental variables were assembled into three data sets: (1) soil climate, (2) biogeochemical characteristics and (3) plant community composition. Soil climate data consisted of average annual soil temperature and soil matric potential. Data was collected using data loggers at each plot and reflect averages between 1988 and 2009. Soil climate was chosen because soil variables more closely reflect the environment saprotrophic fungi experience relative to ambient air temperature and precipitation. The biogeochemical data set consisted of previously collected values of soil pH, forest floor N content (g N m^{-2}), forest floor C:N, leaf litter forest floor turnover (years), and annual ambient N deposition (g N m^{-2} y^{-1}) from 2005 to 2009 (Table 3.1). Plant community composition was calculated as the relative dominance based on basal area of overstory species at the site (Table S3.1). Soil climate and biogeochemical dissimilarity matrices were generated with Euclidean distances of log-transformed data. The plant community dissimilarity matrix was generated using the Bray-Curtis metric (Bray and Curtis, 1957).

Regression analyses were used to investigate the important environmental variables that contributed to differences in fungal phylogenetic β-diversity and functional richness (Anderson et al. 2011, Fine and Kembel 2011, Stegen et al. 2012). Simple and multiple linear regression was used to quantify pairwise community differences attributable to geographic distance (a surrogate for time), plant community composition, as well as soil climate and biogeochemical characteristics across sites. Regression analysis was selected as most appropriate for our data set, as the partial Mantel test underestimates amount of variation in community composition using dissimilarity matrices (Legendre et al. 2005) and we lacked statistical power ($n = 4$) to conduct redundancy or canonical analysis that considered more than one variable at a time. Raw environmental distances were standardized to z-scores prior to regression and Akaike's Information Criterion (AIC) was used to compare linear models. All assumptions of linearity were verified prior to analysis. When there was a significant relationship between an environmental category (*e.g.*, biogeochemical) and phylogenetic or functional distance, we evaluated the relative importance of individual environmental factors (*e.g.*, soil pH) in structuring community differences. Regression was also used to test the relationship between β-diversity and functional composition.

RESULTS

Fungal community composition

Following DNA extraction from forest floor samples, analysis of 1,377 non-chimeric fungal sequences resulted in 721 unique sequences clustering within the subkingdom Dikarya, with an average sequence length of ~ 900 bp. One-hundred-sixty-nine ascomycete and 129 basidiomycete OTUs were identified at 99% 28S sequence similarity. Ascomycete OTUs fell

into the sub-phylum Pezizomycotina, whereas basidiomycete OTUs resided in sub-phylums Agaricomycotina and Pucciniomycotina. Good's coverage values from clone libraries ranged from 80% to 86%, suggesting that the most abundant members of each community were sufficiently sampled; although, some rare community members were not captured by our sampling effort.

Within each site, phylogenetic diversity (PD), net relatedness index (NRI), and nearest-taxon-index (NTI) were calculated (Faith 1992) to characterize phylogenetic composition across our chronosequence. Phylogenetic diversity, the minimum branch length spanning a set of organisms on a phylogenetic tree, ranged from 1.89 to 2.20 among sites, and one-way ANOVA indicated that phylogenetic diversity did not differ among them (Table 3.2; $P = 0.61$). Net Relatedness Index ranged from 1.35 to 3.18 among sites in our chronosequence; it was significantly different from zero in Sites A, C and D ($P < 0.01$), indicative of phylogenetic clustering. However, NRI in Site B was not significantly different from zero ($P = 0.09$). Nearest Taxon Index (NTI) ranged from 0.66 to 3.42, and it was significantly greater than zero in Sites A, B and C ($P < 0.01$) further indicating phylogenetic clustering within each community. The oldest site, Site D, did not exhibit phylogenetic clustering when considering its nearest neighbors, because its NTI was indistinguishable from zero (Table 3.2; $P = 0.260$). In combination, these observations indicated that fungal communities exhibit phylogenetic clustering along the chronosequence, although phylogenetic diversity did not vary among sites.

Phylogenetic differences between communities composing our chronosequence were calculated by β-Nearest Taxon Distance (βNTI) and Rao's phylogenetic distance (D_p). The combination of metrics allowed β-diversity comparisons at two different phylogenetic resolutions (Stegen et al. 2012). For example, βNTI quantified distance only between nearest

relatives, whereas D_p accounted for evolutionary relationships among taxa in both recent and deep evolutionary time. βNTI ranged from 0.71 to 3.65 for all pairwise comparisons of sites, and it was greatest between Sites A and D (the most distant sites) and smallest between Sites C and D (the closest sites; see Table S3.2). Despite variation in βNTI across the chronosequence, D_p narrowly ranged from 0.087 to 0.099 for all pairwise comparisons (Table S3.2), indicating that a small amount of unique branch length distinguished communities in our chronosequence. Taken together, fungal community along the chronosequence differed from one another in the occurrence of closely related species.

Factors structuring phylogenetic β-diversity

We modeled β-diversity as a function of pairwise differences in space (proxy for time) as well as environmental variation across the gradient (Anderson et al. 2011) in order to gain insight into the potential role of dispersal limitation and habitat filtering in structuring fungal composition (Figure 3.2). βNTI was significantly and positively related to log geographic distance ($r^2 = 0.91$, $P = 0.003$, AIC = 7.75) as well as biogeochemical distance ($r^2 = 0.71$, $P = 0.04$, AIC = 14.86); geographic and biogeochemical distances were z-transformed in this analysis. In contrast, βNTI was not related to soil climate ($P = 0.97$) or plant community composition ($P = 0.71$). Despite a weak correlation between geographic and biogeochemical distances ($P = 0.09$, $r^2 = 0.48$, Table S3.5), the combination of spatial (*i.e.,* site age) and biogeochemical factors predicted phylogenetic β-diversity with a higher goodness of fit ($P = 0.01$, $r^2 = 0.95$, AIC = 6.31), than either factor alone in a linear regression model. Independent of factor order in the multiple regression model, the partial multiple regression coefficient for geographic distance did not differ from linear regression coefficient ($\beta = 0.75$, $P = 0.17$), whereas the partial multiple regression coefficient for biogeochemical distance was slightly

lower than that in the linear regression model ($\beta = 0.30$, $P = 0.06$). Results demonstrate that confounding variation in biogeochemical properties does not independently drive the positive relationship between geographic distance, a proxy for site age, and fungal β-diversity. To gain a deeper understanding of the mechanism by which environmental variation structured fungal communities, biogeochemical variables were investigated independently using simple linear regression (Table 3.3). Following correction for false discovery rate (Benjamini and Hochberg 1995), no single biogeochemical variable predicted βNTI ($P = 0.14$ to 0.68).

Functional potential of fungal communities

Using a subset of gene probes on GeoChip 4.0 (*i.e.,* genes synthesizing amylases, cellulases, chitinases, hemicellulases, ligninases, proteases), we quantified the physiological potential of fungal communities to metabolize plant and fungal detritus; these included functional richness, metabolic potential and functional composition. Probe signal intensity was transformed to presence-absence data to calculate functional richness. Two-way ANOVA revealed significant effects of site ($F_{3,48} = 19.40$, $P = 0.002$) and gene category ($F_{5,48} = 4.40$, $P < 0.001$) on functional richness, but there was no significant interaction between them ($F_{14,48} = 0.43$, $P = 0.96$). Post hoc analyses revealed that functional richness, when averaged across gene category, was highest in the oldest site D, followed by site C and site B. However, average functional richness of the youngest site, A, was not significantly different from B (Figure 3.1). As input for regression analysis, a distance matrix was generated using the Sørenson dissimilarity metric, in which the functional richness of each gene category was considered. Pairwise differences in functional richness ranged from 2.6% to 13.0%.

Metabolic potential, calculated as corrected signal intensity (SI), significantly varied between sites (one-way ANOVA; $F_{3,8} = 4.89$, $P = 0.032$), and similarly increased with site age

(r^2 = 0.90, P = 0.049, Figure S3.4). Shapiro-Wilks test of normality indicated data was normally distributed (P = 0.12) and equal variance was confirmed upon inspection of residual plots. A distance matrix of functional composition was generated by the Bray-Curtis dissimilarity metric. Pairwise site differences in functional composition ranged from 0.94% to 2.00%.

Factors structuring community functional potential

We employed linear models to investigate the influence of spatial and environmental factors on the functional richness of fungal genes involved in litter decay. Despite the increasing trend in functional richness across the chronosequence, pairwise site differences in functional richness did not significantly correlate to geographic distance (r^2 =0.03, P = 0.74), biogeochemical distance (r^2 =0.11, P = 0.51), soil climate (r^2 =0.59, P = 0.07) or plant composition (r^2 =0.26, P = 0.31). Because functional composition did not vary across the chronosequence, we did not relate spatial and environmental variation to differences in functional composition.

Despite patterned differences in β-diversity along the chronosequence, the relative abundance of functional genes involved in litter decay did not differ predictably among sites. In addition, functional composition was not related to βNTI (r^2 = 0.13, P = 0.49). Results from multivariate correlation test RELATE further indicated no significant relationship occurred between β-diversity and pairwise differences in functional composition (ρ = 0.26, P = 0.50).

DISCUSSION

While microbial communities are thought to assemble rapidly in the absence of barriers to dispersal, we present evidence suggesting that dispersal limitation is a significant ecological

force shaping saprotrophic fungal communities over the timeframe of centuries to millennia. Evidence for dispersal limitation across our long-term chronosequence comes from the significant relationship we have documented between phylogenetic composition and time since deglaciation, wherein community dissimilarity increased along our spatial proxy for time since glacial retreat. Despite our attempts to minimize the influence of environmental filtering, fungal community dissimilarity also was influenced by biogeochemical variation among sites in our chronosequence. However, environmental filtering was overshadowed by the effect of dispersal limitation as revealed by our analyses. Dispersal limitation may also shape the functional potential of fungal communities, wherein functional richness and metabolic potential both increased with site age.

Ecological factors structuring fungal communities

While fungal spores have the potential to travel long distances (Favet et al. 2012), our findings indicate that temporal differences among sites differentiated fungal communities as a result of restricted dispersal. Phylogenetic differences in saprotrophic fungal communities increased with geographic distance, a surrogate for time since deglaciation, and accounted for more variation in community composition than biogeochemical distance alone (Figure 3.2). One might argue that unmeasured environmental factors could underlie and covary with this relationship. However, our characterization of climate, plant community composition and biogeochemical properties minimizes this possibility. Widespread dispersal in arctic ectomycorrhizal fungi (Geml et al. 2012) indicates that the strength of geographic isolation may depend on habitat and fungal spore characteristics. The open, snow covered arctic landscape potentially enhances spore transport by wind; whereas, tree density in forests may limit long-distance fungal dispersal. Further, extreme environments such as the arctic may select fungi with

long-distance dispersal capacities, such as small spore size and dark pigmentation (Timling & Taylor 2012). Our findings challenge previous hypotheses regarding rapid community assembly and support the idea that restricted dispersal may have a persistent effect on microbial communities through time (Eisenlord et al. 2012).

Dispersal limitation and environmental filtering appear to work together to differentiate fungal communities in our chronosequence. Support for this assertion comes from the combination of time since deglaciation (*i.e.,* distance) and biogeochemical characteristics providing a model with the highest goodness of fit for predicting phylogenetic β-diversity. Physiological adaptations for resource acquisition and defense mechanisms demonstrate that resource competition can influence fungal survival (Lindahl and Olsson 2004, Boddy 2000). Based on fundamental competition theory, organisms that are more efficient in resource capture outcompete and exclude organisms that are less efficient (Tilman 1980). Subtle variation in biogeochemical characteristics across the chronosequence plausibly shaped traits for effective resource competition, assuming no variation or interaction with other factors. Results for environmental filtering are supported by evidence that fungal community composition is structured by biogeochemical factors, including soil pH, ambient N deposition and litter biochemistry (Hazard et al. 2013, Zak et al. 2011, Osono and Takeda 2002).

Historical processes may also govern the functional potential of fungal communities, demonstrated by increasing metabolic potential and functional gene richness across the chronosequence. However, we could not discern whether dispersal limitation or unmeasured environmental factors resulted in functional characteristics. Higher metabolic potential and functional richness in the older sites could be facilitated by adaptive radiation, as nucleotide substitutions created new functional gene variants, thereby increasing physiological diversity.

Interestingly, a direct relationship between phylogenetic diversity and site age was not observed when considering ribosomal genes as molecular markers. However, mutation rates of fungal ribosomal genes occur slowly over evolutionary time (Taylor and Berbee 2006). It is possible that insufficient time has elapsed following deglaciation to allow radiation among colonizing organisms, unlike the evolutionary forces operating on functional genes as described above. Although differences in sampling completeness of the two molecular approaches may provide an alternative explanation, the relatively recent history of our study sites provides a more probable rationale to explain patterns of community richness in functional genes. As a result, older sites may have increased intraspecific variability with respect to the ability to enzymatically harvest energy from plant and fungal detritus.

Functional richness might have consequences for ecosystem processes by providing more opportunities for efficient resource use in a variable environment (*e.g.,* niche complementary effect) or increasing the probability that the presence of a functional trait will dominate ecosystem functioning (*e.g.,* selection effect; Loreau 1998). Empirical support for this idea comes from a grassland ecosystem, in which rates of soil organic matter accumulation were positively related to functional gene richness (Zhang et al. 2007). Our reported increase in metabolic potential and functional richness of the fungal community to degrade plant litter provides further support for this assertion.

Link between community composition & functional potential

Contrary to our predictions, we did not observe a direct link between functional gene composition and fungal β-diversity. One potential explanation is that the collective suite of functional genes involved in litter decay was not phylogenetically conserved among saprotrophic fungi in the soils of our chronosequence. However, certain capabilities including ligninolytic

decay are known to be constrained in saprotrophic fungi of the Dikarya subkingdom (Osono 2007, Baldrian 2006), indicating that functional traits can in fact be conserved for this group of organisms. Metabolically less complex components of litter decay may have evolved independently within saprotrophic fungi. Alternatively, constrained variation in plant communities may explain the lack of correlation between functional gene composition and fungal community composition. By minimizing variation in plant community composition and biogeochemical factors, our experimental design may also have constrained functional composition via the effect of plant litter biochemistry on saprotrophic metabolism. The aforementioned observations do not necessarily dispel the hypothesis that community composition has functional consequences. Instead, community assembly rules may vary based upon level of community organization (Fukami et al. 2005). While our study investigated the links between composition and functional potential, enzyme synthesis via transcription and translation also contributes to biogeochemical processes. Closer investigation of gene regulation and rates of soil C cycling may be required to capture the complete linkage between community composition and function.

Conclusion

In summary, our study suggests that dispersal limitation is an ecological force shaping the composition and functional potential of litter-decay fungi in a long-term chronosequence. The persistent effect of dispersal limitation on fungal community assembly challenges previous conceptions of ubiquitous dispersal and rapid community assembly. Dispersal limitation in ectomycorrhizal fungi (Peay et al. 2010), as well as filamentous bacteria (Eisenlord et al. 2012), for example, adds additional support to the notion that this ecological mechanisms shapes composition and function of various groups of soil microorganisms. Improved knowledge of

fungal dispersal capabilities as well as future manipulative studies are needed to directly test the influence of dispersal limitation on fungal community assembly. Nonetheless, our results highlight the importance of dispersal limitation as an ecological agent shaping community composition and functional traits of saprotrophic fungal communities in temperate forests.

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Table 3.1. Site location, age, soil climate, and biogeochemical soil characteristics.

Site Characteristics	*A*	*B*	*C*	*D*
Location	46:51N; 88:52W	45:32N; 84:51W	44:22N; 85:49W	43:40N; 86:08W
Glacial retreat (yrs before present)	9500	11,000	13,000	13,500
Soil Climate				
Average Soil Temperature (°C)	7.2	7.9	8.3	9.1
Average Soil Matric Potential (MPa)	-0.14	-0.15	-0.14	-0.18
Biogeochemical Characteristics				
Soil pH	4.55	4.70	4.41	4.61
Forest floor N content (g N m^{-2})	14.3	32.1	25.9	40.6
Forest floor C:N	63.7	57.1	52.9	43.4
Forest floor turnover (y)	2.21	4.91	5.16	6.48
Ambient N deposition (kg N ha^{-1} y^{-1})	5.89	6.07	7.37	7.37

Table 3.2. Metrics of phylogenetic community composition computed for each site. PD represents phylogenetic diversity and NTI is Nearest Taxon Index. † indicates significant differences by comparison to a randomly generated phylogeny at $P < 0.01$ and ‡denotes significant differences $P < 0.001$. PD was not significantly different across sites ($P = 0.61$). Significant p-values for NTI and NRI indicate fungal communities are more closely related than null communities.

Site	*PD*	*NRI*	*NTI*
A	1.89	3.18‡	3.42‡
B	2.2	1.35	2.12†
C	1.79	2.28‡	3.17‡
D	2.16	3.11‡	0.66

Table 3.3. Model ranking using Akaike information criterion (AIC), where phylogenetic β-diversity (βNTI) is the response variable. P-values were corrected for false discovery rate using the Benjamini and Hochberg correction.

Model	*AIC*	*P-value*
Annual Ambient N deposition	13.33	0.14
Forest Floor Turnover	15.74	0.18
Litter N	19.55	0.36
Litter C	19.85	0.36
pH	20.13	0.36
Litter C:N	20.65	0.39
Litter Mass	21.94	0.68

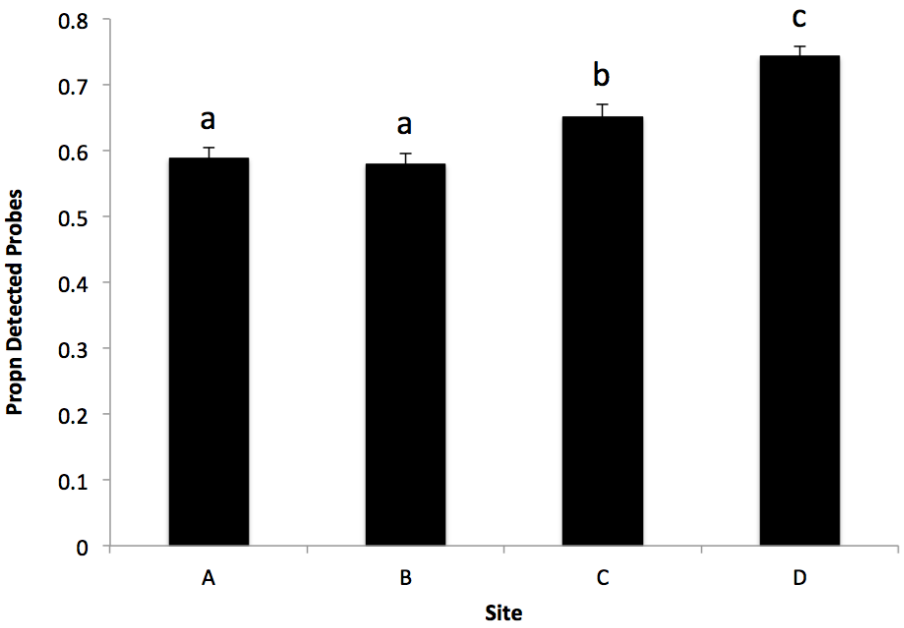

Figure 3.1. Site functional richness, calculated as the average proportion of probes detected across all gene categories. Each functional gene has an equal weight within a gene category. Results of two-way ANOVA indicate significant main effects by site ($F_{3,48} = 19.40$, $P = 0.002$) and gene category ($F_{5,48} = 4.40$, $P < 0.001$), but there was no significant interaction between them ($F_{14,48} = 0.43$, $P = 0.96$). Error bars represent standard error. Lowercase letters represent statistical differences.

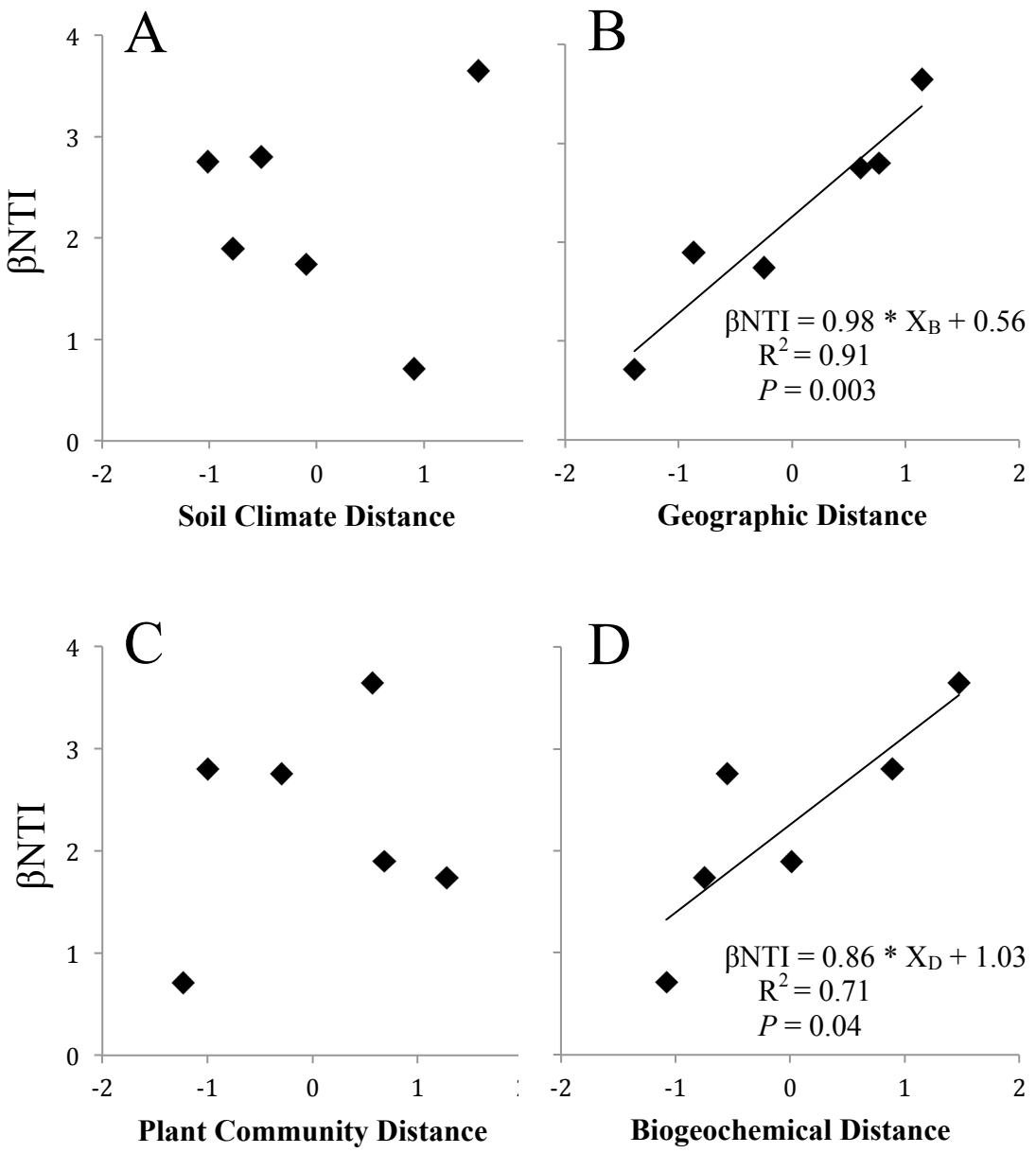

Figure 3.2. Pairwise phylogenetic β-diversity (βNTI) plotted against soil climate distance (A), geographic distance (B), plant community distance (C) and biogeochemical distance (D). All environmental distances were standardized to z-scores for meaningful comparison. Linear regression reveals a significant positive relationship between βNTI and geographic distance, as well as log biogeochemical distance at $P < 0.05$. No significant relationship was found between βNTI and log-transformed soil climate distance ($P = 0.96$) or log-transformed plant community distance ($P = 0.72$).

SUPPLEMENTARY TABLES

Table S3.1. Relative abundance of plant composition based on basal areas of overstory tree species. AB= American Beech, BC= Black Cherry, BF= Balsam Fir, BO= Black Oak, BW = American Basswood, SM = Sugar Maple, RM = Red Maple, RO = Red Oak, IW = Ironwood, YB = Yellow Birch, WA = White Ash.

Site	AB	BC	BF	BO	BW	SM	RM	RO	IW	YB	WA
A	0.000	0.000	0.000	0.000	0.015	0.923	0.053	0.000	0.001	0.008	0.000
B	0.002	0.000	0.000	0.000	0.011	0.868	0.000	0.000	0.000	0.000	0.119
C	0.001	0.012	0.000	0.045	0.000	0.786	0.095	0.061	0.000	0.000	0.000
D	0.020	0.072	0.000	0.000	0.000	0.715	0.149	0.043	0.001	0.000	0.000

Table S3.2. Pairwise site comparisons of phylogenetic composition β-diversity based on two metrics, βNTI and D_p. β-Nearest Taxon Distance (βNTI) calculates the minimum phylogenetic distance between an OTU in one community and all OTUs in a second community and then determines the number of standard deviations from a null model community. βNTI significantly greater than 0 suggests phylogenetic β-diversity is greater than expected for one pairwise comparison and less than 0 suggests lower than expected phylogenetic β-diversity. Rao's phylogenetic distance (D_p) calculated the pairwise differences in branch length of OTUs in a community relative to another. A higher D_p value for a pairwise comparison is indicative of higher phylogenetic distance between sites.

Pairwise site comparison	βNTI	D_p
A-B	2.75	0.091
A-C	2.80	0.091
A-D	3.65	0.087
B-C	1.90	0.092
B-D	1.74	0.093
C-D	0.71	0.099

Table S3.3. Summary of fungal gene categories used in functional gene microarray analysis from the GeoChip 4.0 based on depolymerization of components found in plant litter.

Enzyme (Gene) Name	EC Number	# Gene Variants	Substrate Category
alpha-amylase (AmyA)	3.2.1.1	65	Amylase
glucoamylase	3.2.1.3	53	Amylase
glucose oxidase	1.1.3.4	14	Amylase
cellobiase	3.2.1.21	87	Cellulase
endoglucanase	3.2.1.4	47	Cellulase
exoglucanase	3.2.1.91	136	Cellulase
acetylglucosaminidase	3.2.1.50	13	Chitinase
chitin deacetylase	3.5.1.41	19	Chitinase
cutinase	3.1.1.74	53	Chitinase
endochitinase	3.2.1.14	123	Chitinase
exochitinase	3.2.1.52	8	Chitinase
mannanse	3.2.1.25	14	Chitinase
alpha galactosidase	3.2.1.22	31	Hemicellulase
alpha-L-arabinofuranosidase (ara)	3.2.1.55	63	Hemicellulase
endopolygalacturonase	3.2.1.15	28	Hemicellulase
exopolygalacturonase	3.2.1.67	36	Hemicellulase
pectin lyase (pec)	4.2.2.10	67	Hemicellulase
pectate lyase	4.2.2.2	7	Hemicellulase
pectinesterase (pme)	3.1.11	20	Hemicellulase
rhamnogalacturonan hydrolase (rgh)	3.2.1.171	36	Hemicellulase
xylanase	3.2.1.8	40	Hemicellulase
glyoxal oxidase (glx)	1.4.3.11	40	Ligninase
lignin peroxidase (lip)	1.11.1.14	32	Ligninase
manganese peroxidase (mnp)	1.11.1.13	33	Ligninase
phenol oxidase	1.10.3.2	195	Ligninase
valine dehydrogenase	1.4.1.8	6	Protease
fumarylacetoacetate hydrolase	3.7.1.2	17	Protease
metalloprotease	3.4.24	40	Protease
aspartyl aminopeptidase	3.4.11.21	19	Protease
cysteine protease	3.4.22	4	Protease
serine protease	3.4.21	52	Protease

Table S3.4. Accession numbers and taxonomy of fungal references sequences used for sequence alignment and phylogenetic tree construction. Sequences were accessed from the National Center for Biotechnology Information (NCBI).

Accession #	Subkingdom	Phylum	Subdivision	Class	Order	Family
AF050276	Dikarya	Ascomycota	Incertae sedis	Incertae sedis	Incertae sedis	Incertae sedis
EU998922	Dikarya	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Helotiaceae
HE578059	Dikarya	Ascomycota	Pezizomycotina	Eurotiomycetes	Eurotiales	Trichocomaceae
EU167609	Dikarya	Ascomycota	Pezizomycotina	Sordariomycetes	Diaporthales	Valsaceae
AF050241	Dikarya	Ascomycota	Pezizomycotina	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae
AF178552	Dikarya	Ascomycota	Pezizomycotina	Sordariomycetes	Chaetosphaeriales	Chaetosphaeriaceae
AJ605998	Dikarya	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Clavicipitaceae
FJ904675	Dikarya	Ascomycota	Pezizomycotina	Lecanoromycetes	Ostropales	Stictidaceae
EU167601	Dikarya	Ascomycota	Pezizomycotina	Dothideomycetes	Dothideales	Dothideaceae
EF110615	Dikarya	Ascomycota	Pezizomycotina	Sordariomycetes	Diaporthales	Harknessiaceae
GU727556	Dikarya	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Dermateaceae
EF596821	Dikarya	Ascomycota	Pezizomycotina	Pezizomycetes	Pezizales	Incertae sedis
DQ923534	Dikarya	Ascomycota	Pezizomycotina	Sordariomycetes	Incertae sedis	Incertae sedis
EU552155	Dikarya	Ascomycota	Pezizomycotina	Sordariomycetes	Xylariales	Amphisphaeriaceae
FJ809855	Dikarya	Ascomycota	Pezizomycotina	Pezizomycetes	Pezizales	Tuberaceae
AY853388	Dikarya	Ascomycota	Pezizomycotina	Lecanoromycete	Lecanorales	Lecanoraceae
FJ755255	Dikarya	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	mitosporic Pleosporales
FJ755252	Dikarya	Ascomycota	Pezizomycotina	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae
EU167570	Dikarya	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Didymellaceae
EU040230	Dikarya	Ascomycota	Pezizomycotina	Leotiomycetes	Onygenales	Myxotrichaceae
EU035425	Dikarya	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Venturiaceae
GQ162813	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Atheliales	Atheliaceae
DQ520098	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Cantharellales	Ceratobasidiaceae
FN907915	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Polyporales	Polyporaceae
FJ755224	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae
GU363490	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Cortinariaceae
AM160631	Dikarya	Basidiomycota	Agaricomycotina	Tremellomycete	Tremellales	Tremellaceae

Table S3.4 continued

Accession #	Subkingdom	Phylum	Subdivision	Class	Order	Family
FN907911	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Polyporales	Meruliaceae
AY256710	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae
AM747290	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Incertae sedis	Incertae sedis
FN907912	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Corticiales	Corticiaceae
FN293011	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Marasmiaceae
AM922288	Dikarya	Basidiomycota	Agaricomycotina	Tremellomycetes	Cystofilobasidiales	Cystofilobasidiacea e
HQ604772	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae
JF680988	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Pleurotaceae
DQ873660	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Auriculariales	Hyaloriaceae
FN907916	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Polyporales	Polyporaceae
EU909231	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Trechisporales	Trechispora
AF347080	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Trechisporales	Trechispora
DQ389734	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae
AM900369	Dikarya	Basidiomycota	Agaricomycotina	Tremellomycete	Tremellales	Trichosporonaceae
JF925333	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Peniophoraceae
FM955848	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Thelephorales	Thelephoraceae
AM712245	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Psathyrellaceae
EU118618	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Clavariaceae
HM035080	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Strophariaceae
DQ112630	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Lycoperdaceae
AM922287	Dikarya	Basidiomycota	Agaricomycotina	Tremellomycete	Tremellales	Tremellaceae
EF192211	Dikarya	Basidiomycota	Puccinomycotina	Pucciniomycetes	Pucciniales	Incertae sedis
GQ336996	Dikarya	Basidiomycota	Puccinomycotina	Microbotryomycetes	Leucosporidiales	Leucosporidiaceae
AM160640	Dikarya	Basidiomycota	Ustilaginomycotina	Exobasidiomycetes	Entylomatales	Entylomataceae
FR686941	NA	Glomeromycota	NA	Glomeromycete	Glomerales	Glomeraceae

SUPPLEMENTARY FIGURES

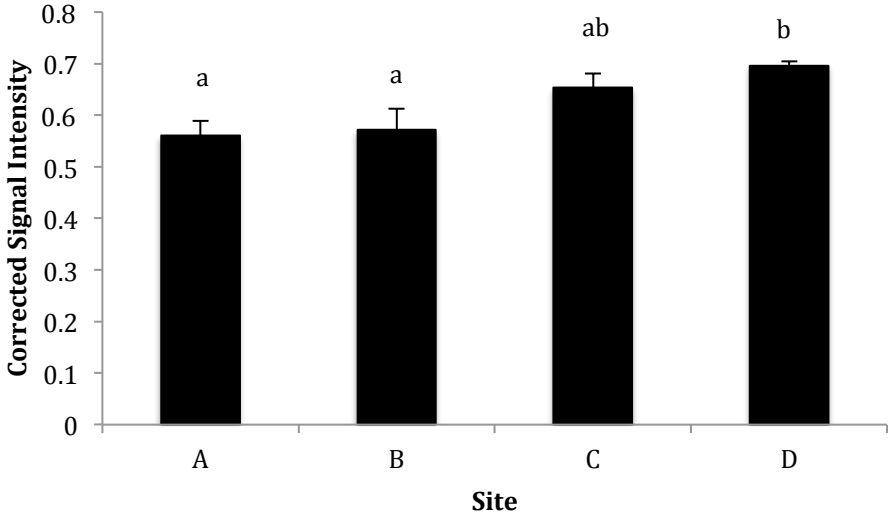

Figure S3.1. Overall metabolic potential, calculated as the corrected fluorescent signal intensity (SI) of all functional gene categories, significantly varied between sites (One-way ANOVA; P = 0.032) and increased with site age. SI was corrected by the number of probes per gene as well as genes per gene category. Lowercase letters represent statistical differences. Error bars represent standard error.

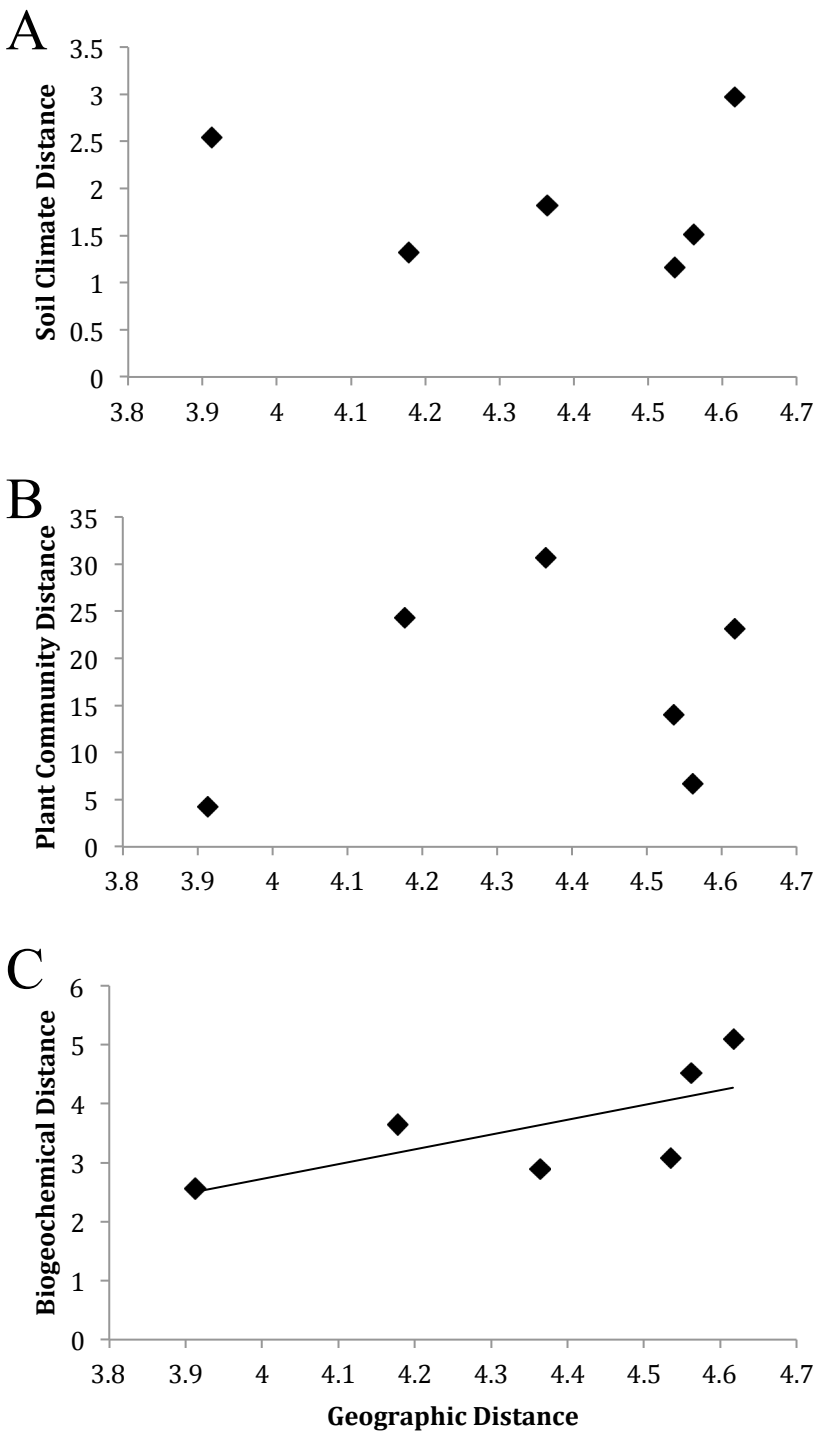

Figure S3.2. Pairwise site comparisons of geographic distance plotted against variation in soil climate (A), plant community composition (B), and biogeochemical characteristics (C). Soil climate and biogeochemical differences calculated from Euclidean distances. Plant community distance calculated from Bray-Curtis dissimilarity metric. A positive trend was observed between pairwise geographic distance and biogeochemical distance. No significant relationship was found between geographic distance and soil climate ($r^2 < 0.001$; $P = 0.95$) or plant community distance ($r^2 = 0.010$; $P = 0.85$).

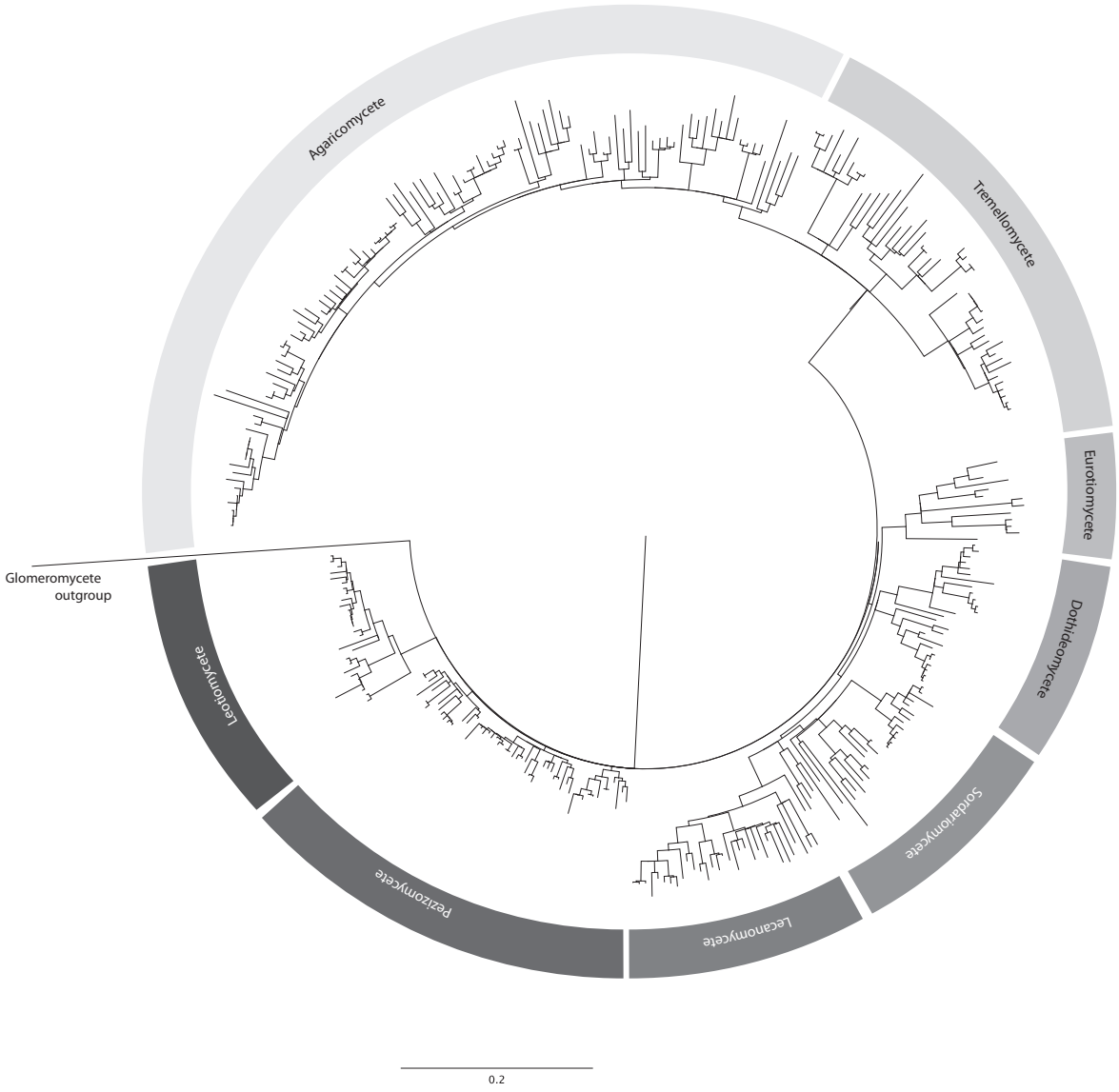

Figure S3.3. Maximum likelihood tree of Dikarya OTUs based on 28S ribsomal gene. Phylogenetic tree was constructed in Mega 5.0 using the general time reversivle (GTR) subsititution method. Taxonomic assignments were based on reference sequences that can be found in Table S3.4 Bar length represents substitution rate.

CHAPTER IV

Resource availability and physiological traits determine strength of priority effects in fungal community assembly

ABSTRACT

The successful establishment of initial colonists may determine the importance of priority effects, thereby shaping the assembly of biotic communities. Using saprotrophic fungi in a model, we investigated whether the strength of priority effects were dependent on initial colonizer traits, resource availability, or a combination thereof. To test these ideas, we factorially manipulated leaf litter biochemistry and initial fungal colonization, quantifying subsequent fungal community composition, metabolic potential, and leaf litter decay. During the first 3 months of our laboratory experiment, trajectories of community assembly were positively related to initial colonist growth rate and metabolic potential to degrade plant detritus. Furthermore, dispersion analysis indicated that energy-poor substrates generated increasingly divergent trajectories of fungal community composition and enzyme potential. Together, our results provide evidence that the strength of priority effects are dependent upon features of habitats and physiological traits within a regional species pool.

INTRODUCTION

Community assembly history, or the stochastic sequence and timing of species arrival, is an important ecological force shaping competitive outcomes, and, in turn, the composition of biotic communities (Lewontin 1969, Diamond 1975, Drake 1991, Chase 2003). Through niche preemption and modification, initial colonizers can exclude later-arriving species via the mechanism known as priority effects (Wilbur and Alford 1985, Belyea and Lancaster 1999, Vannette and Fukami 2014). Furthermore, due to the competitive advantage obtained by initial colonizers, their physiological attributes may have consequences for ecosystem-level processes, including biogeochemical cycling in soils (Fukami et al. 2010, Dickie et al. 2012). Despite our growing knowledge of how historical contingencies shape community assembly, understanding the relative importance of priority effects versus selection imposed by the local environment (habitat filtering) remains unresolved (Chase 2007, Langenheder and Székely 2011, Pagaling et al. 2014); moreover, it may be key to understanding drivers of ecosystem-level processes, especially those mediated by diverse communities of saprotrophic microorganisms in soil (Nemergut et al. 2013).

Local factors that directly impact the success of early-arriving propagules have the potential to alter the balance between habitat filtering and priority effects in shaping community composition and function (Chase 2003, 2007, Ejrnæs et al. 2006, Leopold et al. 2015). For example, the establishment of an initial colonizer depends on the resources available upon arrival. Strong selection for species traits under conditions of low resource supply may decrease the importance of priority effects, functioning as a strong habitat filter, thereby overriding stochastic dispersal effects (Chase 2007). Under high resource availability, initial colonizers have the opportunity to grow quickly and reduce the success of later arriving species. This

situation potentially lessens the impact of habitat filtering on community assembly (Ejrnæs et al. 2006, Kardol et al. 2013). Inasmuch, the importance of priority effects may be dependent upon traits of early-arriving organisms, including resource requirements and growth efficiency (Vannette and Fukami 2014, Cleland et al. 2015). Evidence from yeast and bacteria supports this idea, demonstrating that strong priority effects exist between closely related taxa due to the intensity of competition between ecologically similar species (Peay et al. 2012, Tan et al. 2012).

Elucidating the mechanisms by which priority effects shape competitive outcomes may be critical to understanding the complex relationships between community composition and function (Kardol et al. 2013, Nemergut et al. 2013). However, we do not fully understand how priority effects, habitat filtering, and physiological traits shape the assembly of saprotrophic microbial communities in soil. Moreover, interactions among these factors can plausibly shape rates of ecosystem processes, if compositional differences reflect important functional trait differences in the community. For example, the order of colonization by wood-decay fungi altered rates of decomposition in both laboratory and field settings (Fukami et al. 2010, Dickie et al. 2012), indicating a direct link between community composition and function. Alternatively, if habitat filtering remains a more important ecological force shaping the functional traits of communities, functional convergence may occur during community assembly despite divergence in composition (Fukami et al. 2005).

Using saprotrophic litter-decay fungi as a model system, we implemented a microcosm experiment to investigate how the physiological traits of initial colonizers interact with litter biochemistry, a strong habitat filter, to influence the composition and function of fungal litter-decay communities. First, we reasoned that resource availability shapes competitive outcomes, such that the impact of a particular initial colonizer on community assembly would vary on

lignin-poor and lignin-rich leaf litter. We further reasoned that the importance of an initial colonizer would attenuate with time due to the increased importance of habitat filtering as the biochemical components of plant litter are subsequently metabolized and lignin dominates the latter stages of decay. Lastly, we reasoned that the outcome of priority effects would be similar for closely related fungal taxa that initially colonized leaf litter. We expected that initial colonizers exhibiting rapid growth and/or high lignolytic capacity would result in larger deviations in community composition and function, relative to a "control" community with no initial colonizer. To test these ideas, we factorially manipulated combinations of leaf litter biochemistry and initial fungal colonization to quantify community assembly and leaf decay throughout an eight-month laboratory experiment, the equivalent of a growing season in temperate forests.

METHODS

Experimental design & sampling

To understand the relative importance of the physiological traits of initial fungal colonists and habitat filtering in shaping community assembly, leaves of contrasting biochemistry were collected and litter-decay fungi were cultured from a northern hardwood (NH) ecosystem. This ecosystem was chosen because it is a widespread forest type of North America. Further, previous characterizations of litter-decay fungi at this site provided the background knowledge to experimentally manipulate native, ecologically relevant fungal communities (Edwards and Zak 2011, Entwistle et al. 2013, Cline and Zak 2014).

Leaf litter traps were placed in the field to collect senescent leaves of *Acer saccharum* and *Quercus rubra* (hereafter maple and oak litter). Biochemical analyses revealed that oak leaf

litter had higher lignin and C:N relative to maple litter (Table 4.1). Leaf lignin content was determined by the acid detergent lignin (ADL) procedure, in which ADL was determined gravimetrically as the residue remaining upon ignition after H_2SO_4 treatment (Goering & Van Soest, 1970). Leaf cellulose was calculated by subtracting percent acid detergent fiber (ADF) and lignin from dry mass. ADF was determined gravimetrically as the residue remaining after dissolution and extraction of cell solubles, hemicellulose and soluble minerals with hexadecyltrimethylammonium bromide and sulfuric acid. Total leaf N was measured colorimetrically following digestion in concentrated H_2SO_4 (Lachet Instruments, Loveland, CO).

A fungal isolate collection of dominant litter fungi was generated after sporocarps and decaying leaves were collected from the study site and cultured on malt extract agar plates. Dominant ascomycete saprotrophs that were present in prior molecular inventories of this study site (Edwards and Zak 2011, Entwistle et al. 2013, Cline and Zak 2014), but missing from the culture collection generated here, were obtained from the USDA Forests Products Laboratory. From a collection of 30 fungal isolates, 6 initial colonists were chosen to represent litter-decay fungi with a variety of evolutionary histories and metabolic potentials (Figure 4.1).

Initial colonizers were chosen to represent phylogenetic pairs of fungi, determined by constructing a maximum likelihood phylogenetic tree following DNA extraction and amplification fungal 28S gene (detailed protocols located in the following section). For a list of reference sequences used to construct phylogenetic tree see Table S4.1. Microbial respiration of sterilized oak and maple leaves (see below) was quantified using a gas chromatograph equipped with a Porapak Q column and a thermal conductivity detector (Trace 2000, Thermo Quest, CA). On both litter types, respiration was highest for *Phomopsis* and lowest in *Rhodocollybia*. To

compare the potential enzyme activity of fungal colonists on sterile oak and maple litter, 0.5 g of homogenized leaves were sampled and assayed for the activity of β-1,4-glucosidase, cellobiohydrolase, N-acetyl-β-glucosaminidase, and summed phenol oxidase and peroxidase activity (Saiya-Cork et al. 2002). To measure activity of β-1,4-glucosidase, cellobiohydrolase, N-acetyl-β-glucosaminidase, we used 200 μM methylumbellyferyl MUB-linked substrates. A 25-mM L-dihydroxy- phenlyalanine (L-DOPA) substrate was used to assay phenol oxidase and peroxidase. Enzyme activity was measured in a Molecular Devices f MAX fluorometer set at 365 nm excitation wavelength and 460 nm emission wavelength. Phenol oxidase and peroxidase assays were incubated for 24 h and rates were estimated spectrophotometrically Euclidean distances of log-transformed enzyme activity were calculated to visualize variation in enzyme potential between fungal colonists (Figure 4.1C). PCo1 correlated with β-glucosidase, N-acetylaminoglucosidase and cellobiohydrolase ($r = 0.89 – 0.91$, $P < 0.0001$) and PCo2 correlated with lignolytic activity ($r = - 0.88$, $P < 0.0001$), illustrating the high lignolytic potential of Lachnocladiaceae, as well as the potential of *Phomopsis* to metabolize cellulose and chitin.

To investigate the consequences of niche preemption by an initial colonist and habitat filtering, experimental microcosms were constructed using the two contrasting litter types, which were subsequently inoculated with the initial colonists described above. Microcosms consisted of 250 mL wide-mouth jars containing 2.5 g of maple or oak leaves atop 70 g of acid-washed, autoclaved sand. Leaves were dried at 40 °C, cut into 1 cm^2 squares and sterilized by ethylene oxide fumigation (STERIS, MN). Prior to the experiment, sand was saturated and dry litter was wetted with deionized sterile water. We manipulated initial colonizer history by inoculating a single fungal colonist onto sterile leaves, allowing it to establish (14 days), and then introducing a native saprotrophic community. Importantly, to determine consequences of niche preemption, a

control treatment received no initial colonizer prior to introduction of the native community. Initial colonizers were introduced to each microcosm using two agar plugs from established cultures. Subsequently, the native community was extracted from newly collected decaying litter from our field site. Briefly, 50 g of leaf litter and 500 mL of autoclaved deionized water was homogenized in a blender for 1 min and then filtered through a 500 μm filter to obtain a homogenous suspension. One mL of this slurry was added evenly to each microcosm (He et al. 2010). Microcosms were maintained at 20 °C and 65% water-holding capacity, which is within the favorable range for saprotrophic activity (Langenheder and Prosser 2008). A total of 210 microcosms provided 5 replicates for 2 litter types and 7 initial colonizer histories (including a control), which we harvested at 3 time points. Microcosms were destructively harvested at 1, 3 and 8 months following addition of the native community. At each harvest, leaf mass was determined, after which it was homogenized using sterile scissors. A 0.5 g sample was removed and placed at 4 °C for enzyme assays, whereas the remaining sample was stored at -80 °C for molecular community analysis.

DNA extraction & community analysis

Targeted amplification of the fungal large ribosomal subunit (28S) was performed to characterize community composition. At each harvest time, total DNA was isolated from two replicates of each microcosm using MoBio PowerLyzer DNA Extraction kit. DNA was extracted from 0.25 g of leaf litter and stored at -80 °C, until we could initiate PCR amplification. Fungal β-diversity was estimated by targeting the 28S gene using primers LROR and LR3 (http:// http://sites.biology.duke.edu/fungi/mycolab/primers.htm). Triplicate PCR reactions for each sample contained: 2 μL of DNA, 0.5 μL of 20 μM forward primer, 0.5 μL of 20 μM reverse primer, 0.25 μL dNTPs (20 μM), 2.5 μL 10X PCR buffer (1.5 mM $MgCl_2$; Roche, Hamburg,

Germany), 0.5 µL BSA, 0.5 µL high-fidelity *Taq* polymerase (Roche, Hamburg, Germany), and 18.25 µL molecular-grade water. Following an initial denaturation step at 95 °C for 5 min, PCR was cycled 30 times at 95 °C for 30 s, 54 °C annealing temperature for 30 s, 72 °C for 75 s, and a final extension at 72 °C for 7 min. PCR products were purified using Qiagen MinElute PCR kit and quantified using PicoGreen dsDNA kit.

Sequencing was performed on the PacBio RS II system utilizing circular consensus technology, which can generate 99.5 - 99.9% sequence accuracy for DNA fragments ranging from 150 to 500 bp (Travers et al. 2010). Ten barcoded samples, pooled in equimolar concentrations, were multiplexed on each SMRT chip. Twenty-one total SMRT chips were analyzed at the University of Michigan Sequencing Facility. Sequences were processed in Mothur using established pipeline procedures (Schloss et al. 2011). Sequences aligned to 28S reference alignments (Cole et al. 2014) and chimeras were identified using uchime (Edgar et al. 2011). Each sample was rarefied to 500 sequences; 10 samples failed to meet sequence count and were excluded from the analysis. Operational taxonomic units (OTUs) were clustered at 99% sequence similarity (Martiny et al. 2011) and OTU taxonomic identity was determined using the RDP classifier. In total, 15,181 unique sequences were obtained, ranging in length from 493 to 632 bp. Sequences were assigned to phyla Ascomycota (73.6%), Basidiomycota (19.8%), Fungi *incertae sedis* (6.5%) and a small number of Chytridiomycota (0.1%). The most abundant ascomycete orders consisted of Hypocreales (26%; total sequences) and Eurotiales (24%); whereas, Agaricales (15%) and Polyporales (3%) comprised the most abundant basidiomycete orders. OTU estimates, assessed using Chao1 estimator (Chao 1984, Wang et al. 2007), ranged from 35 to 1597 OTUs per sample. Further, Good's coverage estimates ranging from 0.71 to

0.98 (Good 1953). Following log transformation of OTU relative abundance, taxonomic β-diversity was calculated using the Bray-Curtis dissimilarity metric.

Functional analysis

To characterize litter decomposition, we quantified remaining leaf mass, microbial respiration, and potential enzyme activity. Remaining leaf mass was calculated as ash-free mass remaining after 1, 3 and 8 months of decomposition. Following the addition of the native saprotrophic community to each microcosm, respiration was quantified weekly (according to protocol described above) for the first 3 months of the experiment. After headspace gas was sampled, the lids of the microcosms were removed for 30 min under a sterile hood to equalize CO_2 with the ambient atmosphere. Using the R package grofit, cumulative respiration was fit to the Sigmoidal Gompertz model (Zwietering et al. 1990) to estimate the length of lag time (λ), maximum respiration rate (μ), and amount of substrate available for metabolism (A). Protocols to quantify enzyme potential of litter communities, at 1, 3 and 8 months following source community addition, were described above, and conducted immediately following destructive harvesting. Following log transformation of enzyme potential, pairwise Euclidean distances were calculated for multivariate analysis.

Statistical analysis

A combination of univariate and multivariate statistics were employed to quantify the importance of initial colonizer history, habitat filtering, and physiological traits of colonists in shaping the assembly of saprotrophic microbial communities. To identify whether initial colonizer history resulted in parallel changes in fungal communities and decomposition dynamics, Mantel tests quantified matrix correlations between variation in fungal composition and functional variables. Analysis of variance (ANOVA) determined whether initial colonizer

history and litter biochemistry influenced OTU richness, respiration, and mass loss through time. For fungal β-diversity and potential enzyme activity, we conducted permutational multivariate analysis of variance (PerMANOVA) following 9,999 permutations. To determine whether the impact of initial colonizer history varied across contrasting litter biochemistries, we identified significant interactions between factors in both ANOVA and PerMANOVA models. Partial r^2 values were compared across time points to test the hypothesis that the importance of initial colonization history attenuated through time. Additionally, we employed dispersion analysis for β-diversity and enzyme potential activity to quantify the variability elicited by initial colonizer history between litter biochemistries. Specifically, dispersion was calculated by the average dissimilarity of communities with different fungal colonists to the centroid of all oak and maple litter communities. To evaluate whether outcomes would be similar for closely related colonists, Mantel tests quantified correlations between colonist phylogenetic distance and fungal β-diversity. To test the hypothesis that colonist growth rate and/or lignolytic potential shaped developing communities, linear regression was conducted between colonist characteristics and compositional and functional departures from the control communities; departures from controls were calculated for each litter type, resulting in 12 total comparisons. To aid in community composition analysis, Similarity Percentage analysis (SIMPER) calculated the contribution of OTUs towards the community dissimilarity between each initial colonizer and the control communities. Assumptions of linearity were verified prior to conducting linear regression and ANOVA, followed by necessary transformations. Statistical tests were conducted using the R packages vegan and permdisp2 (http://www.R-project.org).

RESULTS

Initial colonization history shapes community assembly

ANOVA and PerMANOVA results demonstrated that the initial colonizer modified trajectories of fungal community assembly. With respect to the fungal communities developing on oak and maple leaves (hereafter 'oak litter communities' and 'maple litter communities'), the initial fungal colonizer significantly influenced β-diversity, litter decay and enzyme potential at each time point (Table 4.2). Averaged across litter type, the highest OTU richness occurred in litter communities initially colonized with *Rhodocollybia*, followed by the control, then *Gymnopus*, *Aspergillus*, *Phomopsis*, Lachnocladiaceae and finally *Mycena*. Tukey's HSD revealed that oak litter communities initially colonized by *Mycena* had a significantly lower average richness (126 $\pm$ 95 OTUs), relative to the control community growing on oak litter (629 $\pm$ 151 OTUs) after 3 months ($P = 0.012$); no other treatments significantly differed from control communities on oak or maple litter. Across both litter types, the wide range of community dissimilarity (Bray-Curtis) relative to control communities indicated that outcomes of priority effects were dependent on the identity of initial colonizer. For example, the initial colonization of *Mycena* consistently resulted in large community turnover from the control communities growing on oak and maple litter; whereas, colonization by *Rhodocollybia* did not alter community composition (Figure 4.2). Not surprisingly, taxonomic assignment of OTUs contributing to differences in community composition between control and initial colonizer treatments (SIMPER) indicated that initial colonists were more abundant after one month, relative to control communities for each initial colonizer treatment and litter type (Table S4.2). Third, the presence of certain initial colonizers enhanced rates of oak and maple litter decomposition relative to their respective control community. For example, initial colonization

by *Phomopsis* significantly decreased the lag phase of respiration (Table S4.3), indicating this colonist resulted in most rapid initial litter decay. Further, oak litter communities initially colonized by *Gymnopus, Mycena* and Lachnocladiaceae had higher maximum rates of respiration (μ), a greater substrate pool (A), and a greater rate of decay as revealed by litter mass loss (Table S4.3, Figure 4.3). Similarly, maple litter communities inoculated with *Mycena* had significantly larger pools of metabolizable substrate and a lower remaining litter mass, relative to the control community growing on maple litter. Finally, lignolytic potential was enhanced in oak litter communities initially colonized with *Gymnopus, Mycena,* and Lachnocladiaceae, as well as maple litter communities colonized with *Mycena*, as indicated by distinct separation of PCo2 in Figure 4.4C, an axis negatively correlated with lignolytic activity ($r = -0.99$, $P < 0.0001$). Together, results indicate that initial colonists, particularly basidiomycetes with high lignolytic potential, resulted in diverging community composition and enhanced litter decay.

To understand whether initial colonizer history had a consistent effect on fungal community and functional characteristics, we conducted Mantel correlation tests between β-diversity, mass loss, and enzyme potential of oak and maple litter communities. While no significant correlation occurred between variation in mass loss and β-diversity during the first and third months of the experiment ($P = 0.25 - 0.56$), pairwise distance matrices were significantly correlated following 8 months ($R_{Mantel} = 0.26$, $P = 0.045$). This result indicated that the initial fungal colonizer had consistent effects on community composition and metabolic rate during late stages of decay. Further, weak correlations occurred between β-diversity and variation in enzyme potential after 1 month ($R_{Mantel} = 0.13$, $P = 0.098$) and 8 months ($R_{Mantel} = 0.23$, $P = 0.054$); whereas, no significant correlation occurred after 3 months ($P = 0.23$). Overall,

evidence supports our hypothesis that changes to fungal communities, as a result of initial colonization, resulted in corresponding consequences to litter decay.

Litter biochemistry alters consequences of initial colonizer history

Consequences of initial colonization on subsequent fungal community composition and function were dependent on litter biochemistry, as indicated by the significant interaction terms for models of fungal β-diversity, litter decay, and enzyme potential (Table 4.2). Dispersion analysis indicated that litter community composition was more variable on oak leaves relative to maple litter after 3 months (Figure 4.2B, Pseudo-$F_{1,68}$ = 5.73, P = 0.019) and 8 months (Figure 4.2C, Pseudo-$F_{1,58}$ = 5.82, P = 0.019). Similarly, the initial fungal colonizer had a larger effect on the enzyme potential of oak litter communities, demonstrated by the significantly greater dispersion in enzyme potential of oak litter communities relative those growing on maple litter at each time point (Figure 4.4, Pseudo-F = 4.91 - 13.8, $P < 0.001$). Lastly, initial colonization by lignolytic fungi (*i.e.*, *Gymnopus, Mycena* and Lachnocladiaceae) enhanced maximum rates of respiration (μ), substrate pool size (A), and decay rate (following 8 months) on oak litter, although only minor enhancements were observed on maple leaves (Table S4.3, Figure 4.3). These observations collectively indicated that the initial fungal colonizer had a significantly larger effect on community assembly on lignin-rich plant litter.

Role of initial colonizer history on community assembly through time

To test the hypothesis that the importance of initial colonization attenuated through time, we compared partial r^2 values for compositional and functional characteristics of fungal communities at 1, 3 and 8 months (Table 4.2). First, the initial colonist accounted for substantial and relatively consistent variation in fungal β-diversity. Whereas, the importance of the initial colonizer on enzyme potential declined, suggesting that the initial colonist was less important in

determining trajectories of metabolic potential through time. Interestingly, the initial fungal colonist appeared increasingly important during litter decay, as identity of the initial colonist accounted for increasing variance of mass loss through time. Despite the substantial role of initial colonizer in shaping community composition, results indicated functional consequences are dependent on stage of community assembly.

Physiological characteristics of initial colonist and consequences to community assembly

Mantel correlations tested the hypothesis that initial colonization of closely related fungal taxa would result in the assembly of similar communities over time. Following one month of community assembly, β-diversity of maple litter communities was significantly related to pairwise phylogenetic distances between fungal colonists ($R_{Mantel} = 0.62$, $P = 0.032$), but not in oak litter communities ($R_{Mantel} = 0.40$, $P = 0.14$). Conversely, after 3 months, phylogenetic distance between initial colonists was marginally correlated with β-diversity in oak litter communities ($R_{Mantel} = 0.33$, $P = 0.08$), but not maple litter communities ($R_{Mantel} = 0.23$, $P = 0.24$). Finally, after 8 months, variation in colonist phylogenetic distance was not related to the β-diversity of oak litter communities ($R_{Mantel} = - 0.09$, $P = 0.54$) or maple litter communities ($R_{Mantel} = 0.32$, $P = 0.20$). Although phylogenetic relatedness between colonists was not a perfect predictor of community assembly trajectories, phylogenetically similar colonists resulted in more similar communities when compared to distantly related colonists at early stages of decay.

To investigate if particular physiological traits of initial colonist shaped community assembly, respiration, total enzyme activity, and lignolytic activity of colonists were regressed against fungal community compositional and functional departures from the control. Across both litter types, initial colonists with higher rates of respiration (log transformed) correlated with larger Bray-Curtis dissimilarities following one month (Figure 4.5, $r^2 = 0.54$, $P = 0.007$),

although no relationship occurred at later time points (P = 0.17 - 0.47). Further, total enzyme potential or lignolytic potential of initial colonists was not related to community dissimilarity at 1, 3 or 8 months (P = 0.16 - 0.94). Total enzyme potential of initial colonizer was weakly correlated to mass loss (normalized to the control) after 1 month (r^2 = 0.28, $F_{1,10}$ = 3.93, P = 0.076) and 3 months (r^2 = 0.27, $F_{1,10}$ = 3.65, P = 0.085), but not 8 months (P = 0.16). Similarly, variation in community enzyme potential correlated to pairwise differences in colonist enzyme potential after 1 month (R_{Mantel} = 0.28, P = 0.033), although no correlation was observed after 3 and 8 months (P = 0.22 - 0.52). Together, results demonstrate that colonist respiration and total enzyme potential shaped early trajectories of community assembly.

DISCUSSION

Priority effects had important consequences for fungal community assembly, wherein the physiological traits of the initial colonist accounted for the early trajectories of community composition and rates of litter decay. Support for this claim comes from evidence that the initial fungal colonist suppressed fungal community richness and enhanced litter decay, although the degree of divergence was highly dependent on the colonist's identity. Similarly, the initial colonist generated divergent trajectories of community composition and metabolic potential relative to control communities lacking an initial colonist. Furthermore, during the early stages of community assembly (1 and 3 months), deviations from control community assembly were positively related to colonist growth rate and metabolic potential to degrade plant detritus. Importantly, lignin-rich leaf litter generated increasingly divergent trajectories of community assembly, as initial colonizer identity resulted in a broader range of community composition and enzyme potential in oak litter communities relative to maple litter communities. Together, our

results indicate the important roles that physiological traits of initial colonist, as well as resource availability, play in shaping the balance between habitat filtering and priority effects during the process of community assembly.

Initial colonization altered community assembly

Initial colonizer identity altered compositional and functional trajectories of fungal community assembly, indicating that priority effects have important implications for biogeochemical cycling in soils. Providing support for this assertion, models of fungal β-diversity indicated that initial colonizer history accounted for litter community turnover throughout the experiment (Table 4.2). Further, direct evidence for priority effects arose from increased initial colonizer abundance, relative to control communities (1 month, Table S4.2). Gaining early access to resources plausibly enhanced establishment success of fungal colonists, as the absence of competition did not require the production of energetically expensive secondary metabolites necessary for combative interactions (Holmer and Stenlid 1997, Boddy 2000, Dickie et al. 2012), leading to niche preemption. However, initial colonists did not rank top OTUs driving differences between initial colonization treatments and control communities after 3 and 8 months of community assembly. Therefore, lasting consequences of initial colonization were not the result of high initial colonist abundance. Instead, initial colonizer identity appeared to shape trajectories of community assembly by the subsequent suppression or enhancement of later propagule establishment (Fukami et al. 2010, Dickie et al. 2012).

Our results confirmed the hypothesis that characteristics of the initial fungal colonizer significantly altered litter decay and community functional potential (Table 4.2), thereby providing evidence that the priority effects of saproptrophic fungi have important functional implications (Fukami et al. 2010, Dickie et al. 2012). First, initial colonizer identity altered

functional characteristics in a manner that was generally consistent with changes in community composition, indicating that fungal communities are not functionally redundant (McGuire et al. 2010, Kivlin and Treseder 2014). Secondly, litter decomposition appeared sensitive to the strength of priority effects, as functional divergence from control communities was dependent on the identity of the initial colonizer (Cleland et al. 2015). For example, certain initial colonists (*i.e., Gymnopus, Mycena,* and Lachnocladiaceae) led to enhanced respiration and decomposition (Table S4.3, Figure 4.3), with largest differences in enzyme activity apparent in communities initially colonized by lignolytic fungi (Figure 4.4). While some observations indicated that functional characteristics may converge despite strong priority effects (*e.g.,* Fukami et al. 2005, Petermann et al. 2010, Tan et al. 2012), our results indicate that the competitive advantage gained by initial fungal decomposers had important consequences for soil biogeochemical cycling and further necessitates investigation of the factors that strengthen priority effects.

Impact of initial colonizer decreases over time

While the initial fungal colonizer shaped community assembly and litter decay throughout the experiment, habitat filtering may become increasingly important at later stages of community assembly (Ferrenberg et al. 2013). Despite accounting for a relatively stable amount of variance in fungal β-diversity and mass loss, the identity of initial colonizer explained less variation in enzyme potential through the time. Concomitantly, litter biochemistry captured an increasing variation in enzyme potential through time, potentially indicating the growing importance of habitat filtering in shaping functional characteristics of communities. Specifically, the depletion of labile organic substrates may increase selection for organisms with the physiological capacity to decompose the lignified components of plant detritus (Hudson 1968, Frankland 1998, Lonardo et al. 2013). Secondly, the mechanism by which initial colonist shaped

community assembly may change with time, as initial colonist respiration and enzyme activity significantly accounted for deviations from community assembly at early time points (Figure 4.5). In early stages of community assembly, initial colonists may directly influence community traits simply due to their high abundance; whereas, in later stages initial colonizers may indirectly influence community assembly by altering establishment of later-arriving colonizers via prior resource consumption and subsequent niche modification ('impact niche'; Vannette and Fukami 2014). The persistent influence of initial colonizer history indicates that initial colonizers alter the competitive dynamics of later establishing taxa, even after their direct influence dissipates.

Litter biochemistry functions as a habitat filter, altering the influence of the initial fungal colonist

Contrary to our hypothesis, consequences of initial fungal colonizers were stronger on lignin-rich (energy-poor) plant detritus. Supporting this idea, dispersion analysis revealed that oak litter community composition was more variable, indicating initial colonizers elicited larger departures from control community (Figure 4.2B-C). Stronger consequences of initial fungal colonizers were expected on maple leaves, due to observations in which high resource conditions enhanced the establishment success of plant initial colonists and led to increasingly divergent trajectories of community assembly (Ejrnæs et al. 2006, Kardol et al. 2013). Similarly, drought reduced the importance of priority effects as plant taxa were removed according to tolerance to harsh conditions (Chase 2007, Leopold et al. 2015). Because our experimental 'low resource' environment generated wider ranges of community assembly, we believe it is important to recognize that resource availability is highly dependent on the physiological attributes of the organisms under consideration. Although lignin-rich environments may be considered a limited

resource substrate to a sugar fungus, it represents an abundant resource for lignolytic fungi (Osono and Takeda 2001, Voříšková and Baldrian 2013) which may enhance the establishment success of relatively rare or slow-growing taxa, thereby increasing community divergence and subsequent decomposition (Pagaling et al. 2014). Together, our observations indicate that the interactions between habitat filtering and initial colonization determine outcomes of fungal communities developing on leaf litter.

Community assembly related to physiological traits of initial colonist

Consistent with our hypothesis, evolutionary history, growth rate, and enzyme potential of initial colonizers appeared important determinants of community assembly. First, initial colonization by close phylogenetic relatives resulted in similar competitive outcomes when considering subsequent fungal community composition, indicating that the phylogenetic context may be useful to understanding consequences of priority effects on microbial community composition and function (Peay et al. 2012, Tan et al. 2012). Secondly, community assembly during the earliest stage of decomposition appeared dependent on growth rate of the initial colonizer (Figure 4.5), indicating that rapidly growing colonists, such as *Phomopsis*, gained a competitive advantage in early stages of community assembly. Third, the total enzyme potential of initial colonizers weakly correlated with mass loss (normalized to control) after one and three months. Although not statistically significant ($P = 0.076 – 0.085$), this relationship provides support for the idea that metabolic potential of initial colonists altered decomposition rates, as a result of changes in community composition. Interestingly, the largest departures in community composition and litter decay arose following initial colonization by *Mycena, Gymnopus* and Lachnocladiaceae, all basidiomycetes capable of decomposing lignin. While no linear relationship occurred between colonist lignolytic potential and community departure from

control communities, our observations indicate that ability to metabolize lignin may be one of several factors that determine strength of priority effects in saprotrophic communities.

Conclusion

The importance of the stochastic sequence and timing of propagules may hinder our ability to predict outcomes of community assembly. Here, we have demonstrated that an initial colonizer can alter the community composition and functional characteristics of assembling saprotrophic fungi, as a result of priority effects. However, we also present evidence that strength of these effects may be dependent upon critical features of habitats and physiological traits within a regional species pool. As a result, identifiable ecological mechanisms appear to underlie the seemingly stochastic consequences of priority effects. Investigation of the factors that alter dispersal and establishment success of organisms is necessary for a comprehensive understanding of factors that influence strength of priority effects, and ultimately, the factors that structure community assembly, as well as ecosystem-level responses.

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Table 4.1. Litter biochemistry of *Acer saccharum* and *Quercus rubra* leaves collected for use in microcosm experiment.

	Acer saccharum	*Quercus rubra*
Lignin (mg g^{-1})	118	263
Cellulose (mg g^{-1})	251	240
Leaf C:N	41:1	62:1

Table 4.2. Partial r^2 values from ANOVA and PerMANOVA models at each experimental time point. C*L indicates interaction term between colonizer and litter type. *** represents factor significance at $\alpha < 0.001$, ** denotes $\alpha < 0.01$, * denotes $\alpha < 0.05$, ^ denotes $\alpha < 0.10$.

	Factor	1 Month	3 Months	8 Months
OTU Richness	Colonizer	0.23^{**}	0.51^{***}	0.24^{**}
	Litter Type	0.05^{*}	0.01	0.06^{*}
	C x L	0.16^{*}	0.05	$0.15^{\wedge}$
β-diversity	Colonizer	0.25^{***}	0.22^{***}	0.21^{***}
	Litter Type	0.04^{***}	0.06^{***}	0.08^{***}
	C x L	0.11^{***}	0.09^{***}	0.11^{***}
Enzyme potential	Colonizer	0.39^{***}	0.25^{***}	0.21^{***}
	Litter Type	0.23^{***}	0.30^{***}	0.47^{***}
	C x L	0.09^{**}	0.09^{*}	0.11^{**}
Mass Loss	Colonizer	0.10^{*}	0.22^{***}	0.20^{**}
	Litter Type	0.43^{***}	0.25^{***}	0.20^{**}
	C x L	$0.09^{\wedge}$	0.12^{*}	0.12^{**}

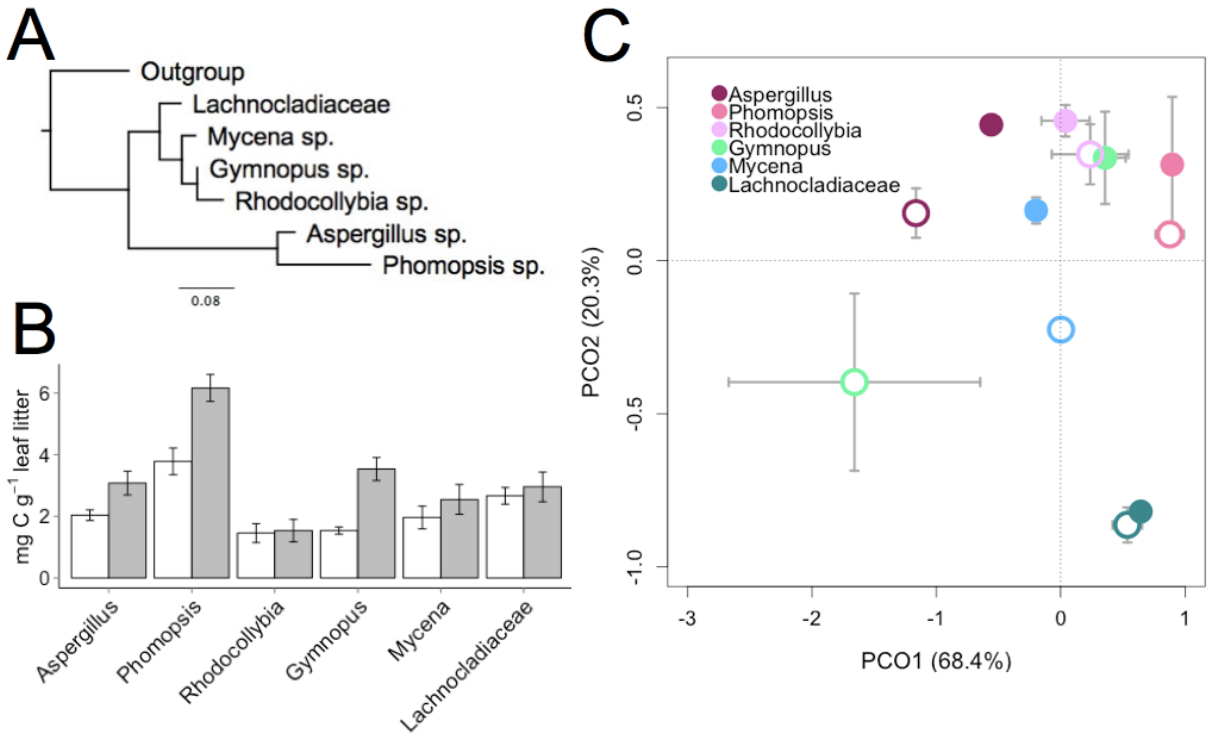

Figure 4.1. Six initial colonizers were characterized and selected according to varied evolutionary histories (A), rates of respiration (B) and potential enzyme activity (C). Maximum likelihood phylogenetic tree was constructed following the amplification of a fragment of the 28S fungal gene. Prior to the inoculation of the litter community, respiration of initial colonizer was quantified using a gas chromatogram (n = 15) and extracellular enzyme assays were conducted (n = 3) on sterile maple and oak leaf litter. Potential enzyme activity was log-transformed followed by the calculation of pairwise Euclidean distance between samples and principal coordinates analysis (PCoA). Maple litter treatments are represented by open bars (B) and open circles (C); whereas, oak litter treatments are denoted by closed bars and closed circles. Error bars represent standard error between replicates within a treatment.

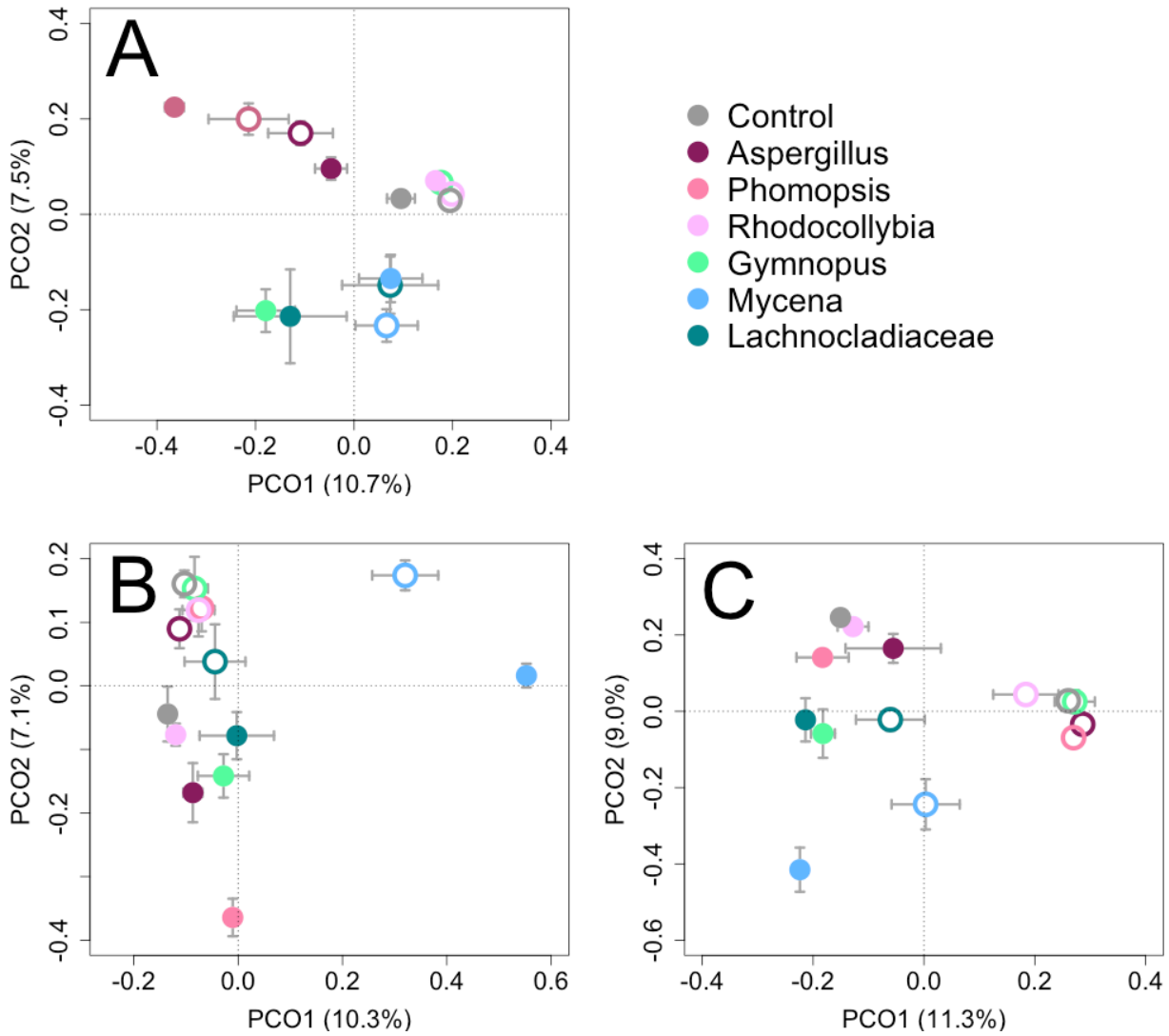

Figure 4.2. Principal components analysis of fungal OTU β-diversity after 1 month (A), three months (B), and eight months (C). The Bray-Curtis distance metric was used to calculate pairwise treatment differences in log-transformed OTU abundances. Open circles represent maple litter communities and closed circles denote oak litter communities. Error bars denote standard error between replicates within a treatment.

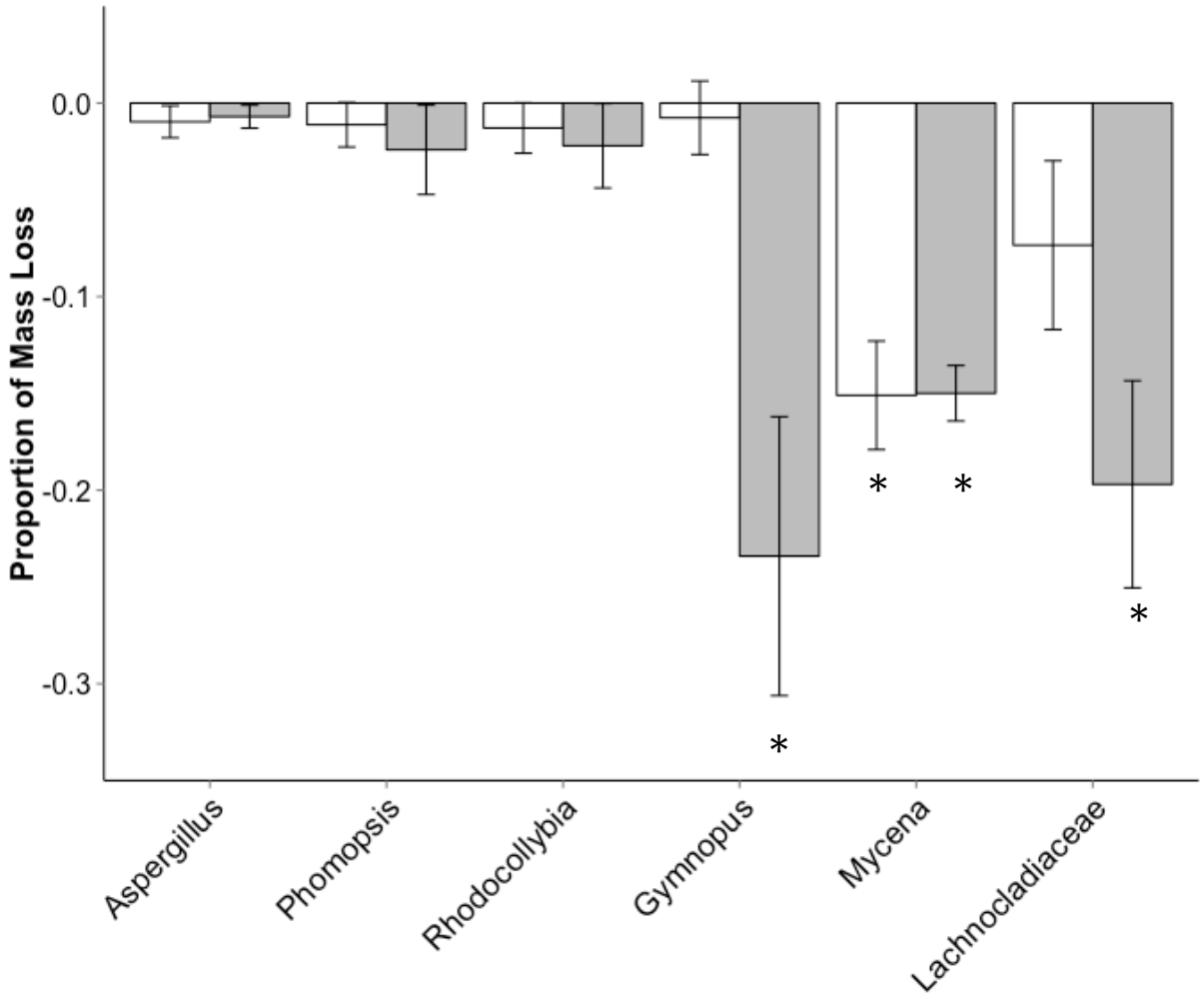

Figure 4.3. Mass loss normalized to the control community on maple (open bars) and red oak (closed bars) litter. Negative values indicate greater mass loss in litter communities inoculated with an initial colonizer. Error bars denote standard error. An asterisk represents significance at $\alpha < 0.05$; whereas, a dot represents $0.05 < \alpha < 0.10$.

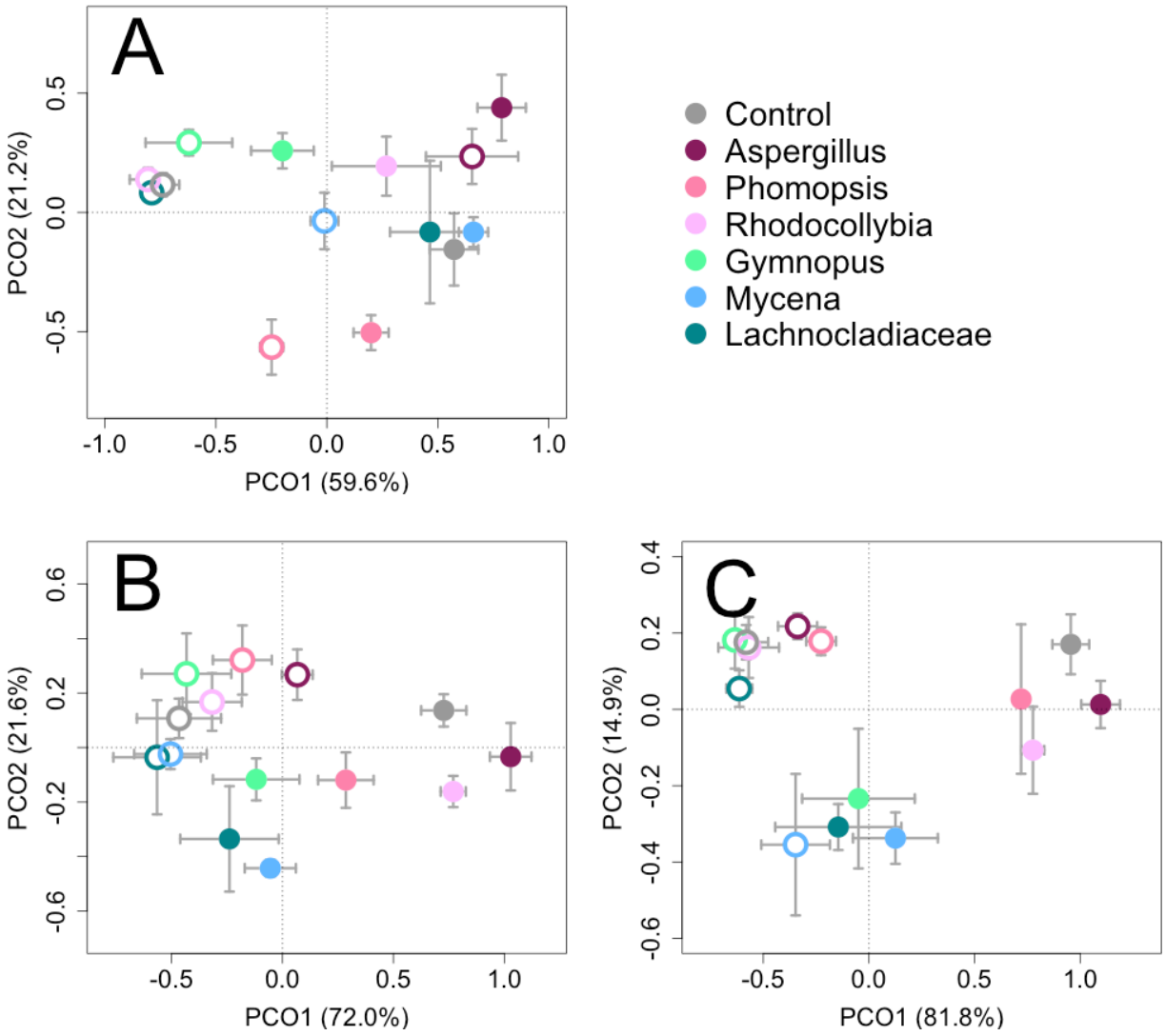

Figure 4.4. Principal components analysis of potential enzyme activity after 1 month (A), 3 months (B) and 8 months (C). The Euclidean distance metric was used to calculate pairwise treatment differences in log-transformed enzyme potential. Open circles represent enzyme potential of maple litter communities and closed circles represent enzyme potential of oak litter communities. Error bars denote standard error between replicates within a treatment. Across all time points, PCo1 negatively correlated with β-glucosidase, N-acetylaminoglucosidase and cellobiohydrolase across all time points (r = -0.69 to -0.93, $P < 0.0001$). PCo2 in plot C is negatively correlated with lignolytic activity (r = -0.99; $P < 0.0001$).

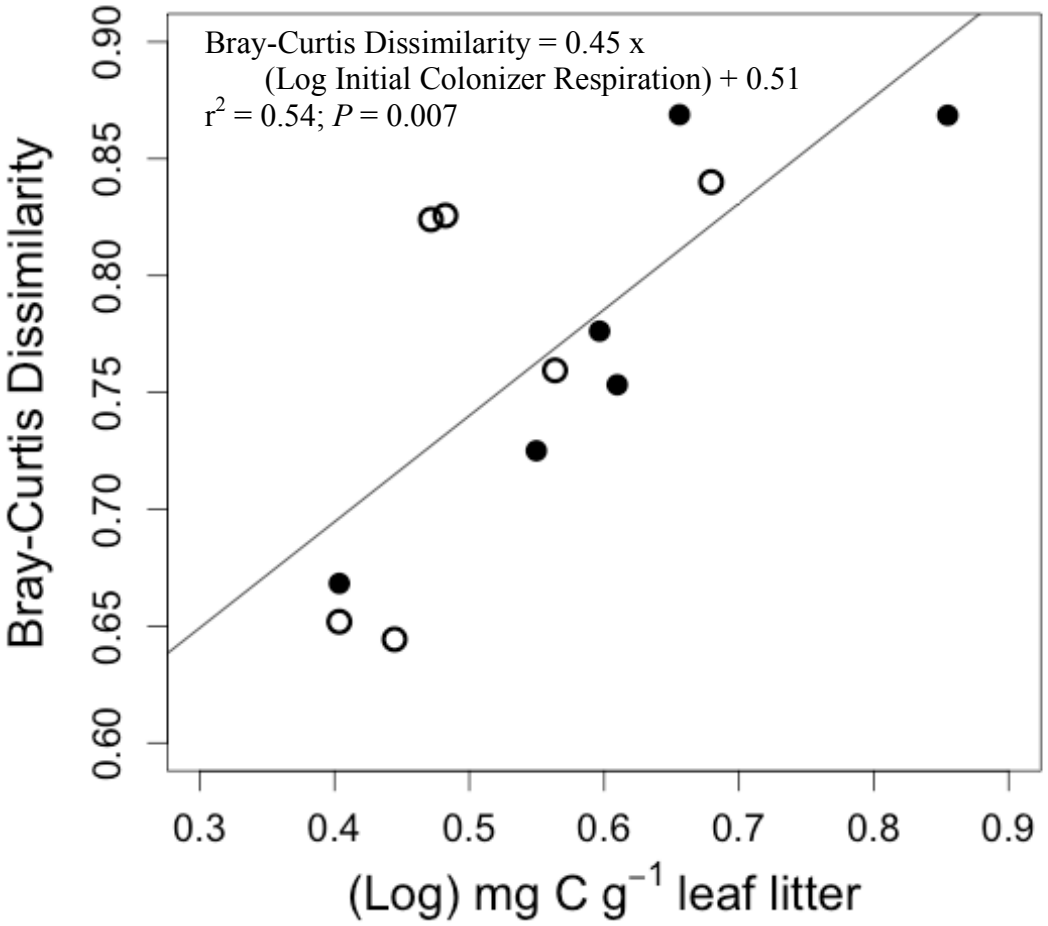

Figure 4.5. Average Bray-Curtis dissimilarity of each initial colonizer history after one month, normalized to control, as a function of (log-transformed) respiration rate of initial colonizer. Simple linear regression revealed a significant relationship at $\alpha < 0.05$. Open circles represent Bray-Curtis dissimilarities of maple litter communities; whereas, closed circles denote pairwise comparisons between oak litter communities.

SUPPLEMENTARY TABLES

Table S4.1. Accession numbers and taxonomy of fungal references sequences used for sequence alignment and phylogenetic tree construction. Sequences were accessed from the National Center for Biotechnology Information (NCBI)

Accession #	Subkingdom	Phylum	Subdivision	Class	Order	Family
AF050276	Dikarya	Ascomycota	Incertae sedis	Incertae sedis	Incertae sedis	Incertae sedis
EU998922	Dikarya	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Helotiaceae
HE578059	Dikarya	Ascomycota	Pezizomycotina	Eurotiomycetes	Eurotiales	Trichocomaceae
EU167609	Dikarya	Ascomycota	Pezizomycotina	Sordariomycetes	Diaporthales	Valsaceae
AF050241	Dikarya	Ascomycota	Pezizomycotina	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae
AF178552	Dikarya	Ascomycota	Pezizomycotina	Sordariomycetes	Chaetosphaeriales	Chaetosphaeriaceae
AJ605998	Dikarya	Ascomycota	Pezizomycotina	Sordariomycetes	Hypocreales	Clavicipitaceae
FJ904675	Dikarya	Ascomycota	Pezizomycotina	Lecanoromycetes	Ostropales	Stictidaceae
EU167601	Dikarya	Ascomycota	Pezizomycotina	Dothideomycetes	Dothideales	Dothideaceae
EF110615	Dikarya	Ascomycota	Pezizomycotina	Sordariomycetes	Diaporthales	Harknessiaceae
GU727556	Dikarya	Ascomycota	Pezizomycotina	Leotiomycetes	Helotiales	Dermateaceae
EF596821	Dikarya	Ascomycota	Pezizomycotina	Pezizomycetes	Pezizales	Incertae sedis
DQ923534	Dikarya	Ascomycota	Pezizomycotina	Sordariomycetes	Incertae sedis	Incertae sedis
EU552155	Dikarya	Ascomycota	Pezizomycotina	Sordariomycetes	Xylariales	Amphisphaeriaceae
FJ809855	Dikarya	Ascomycota	Pezizomycotina	Pezizomycetes	Pezizales	Tuberaceae
AY853388	Dikarya	Ascomycota	Pezizomycotina	Lecanoromycete	Lecanorales	Lecanoraceae
FJ755255	Dikarya	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	mitosporic Pleosporales
FJ755252	Dikarya	Ascomycota	Pezizomycotina	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae
EU167570	Dikarya	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Didymellaceae
EU040230	Dikarya	Ascomycota	Pezizomycotina	Leotiomycetes	Onygenales	Myxotrichaceae
EU035425	Dikarya	Ascomycota	Pezizomycotina	Dothideomycetes	Pleosporales	Venturiaceae
GQ162813	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Atheliales	Atheliaceae
DQ520098	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Cantharellales	Ceratobasidiaceae
FN907915	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Polyporales	Polyporaceae

Table S4.1 continued

Accession #	Subkingdom	Phylum	Subdivision	Class	Order	Family
FJ755224	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae
GU363490	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Cortinariaceae
AM160631	Dikarya	Basidiomycota	Agaricomycotina	Tremellomycete	Tremellales	Tremellaceae
FN907911	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Polyporales	Meruliaceae
AY256710	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae
AM747290	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Incertae sedis	Incertae sedis
FN907912	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Corticiales	Corticiaceae
FN293011	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Marasmiaceae
AM922288	Dikarya	Basidiomycota	Agaricomycotina	Tremellomycetes	Cystofilobasidiales	Cystofilobasidiacea e
HQ604772	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae
JF680988	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Pleurotaceae
DQ873660	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Auriculariales	Hyaloriaceae
FN907916	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Polyporales	Polyporaceae
EU909231	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Trechisporales	Trechispora
AF347080	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Trechisporales	Trechispora
DQ389734	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Tricholomataceae
AM900369	Dikarya	Basidiomycota	Agaricomycotina	Tremellomycete	Tremellales	Trichosporonaceae
JF925333	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Russulales	Peniophoraceae
FM955848	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Thelephorales	Thelephoraceae
AM712245	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Psathyrellaceae
EU118618	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Clavariaceae
HM035080	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Strophariaceae
DQ112630	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricales	Lycoperdaceae
AM922287	Dikarya	Basidiomycota	Agaricomycotina	Tremellomycete	Tremellales	Tremellaceae
EF192211	Dikarya	Basidiomycota	Puccinomycotina	Pucciniomycetes	Pucciniales	Incertae sedis
GQ336996	Dikarya	Basidiomycota	Puccinomycotina	Microbotryomycetes	Leucosporidiales	Leucosporidiaceae
AM160640	Dikarya	Basidiomycota	Ustilaginomycotina	Exobasidiomycetes	Entylomatales	Entylomataceae
FR686941	NA	Glomeromycota	NA	Glomeromycete	Glomerales	Glomeraceae

Table S4.2. Individual contribution of OTUs towards the pairwise community dissimilarity between each initial colonizer history and the respective control community. Calculated by similarity percentage analysis (SIMPER) for each time point (1, 3 and 8 months) and litter type (oak and maple). Taxonomic assignment of OTUs was conducted using the Ribosomal Database Project (RDP) classifier. (+) indicates an OTU was more abundant in the litter community receiving an initial colonist relative to the control community; whereas, (-) indicates abundance was higher in the control community.

Time	Litter Type	Inocula	Contributing Difference (Propn)	Abund	Phylum	Class	Order	Family	Genus
1 mo	Maple	Aspergillus	0.024	(+)	Ascomycota	*Eurotiomycetes*	Eurotiales	Trichocomaceae	Aspergillus
			0.018	(-)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.016	(-)	Ascomycota	*Eurotiomycetes*	Eurotiales	Trichocomaceae	Pilidium
			0.012	(+)	Ascomycota	Leotiomycetes	Heliotiales	Helotiales_incertae_sedis	unclassified
		Phomopsis	0.029	(+)	Ascomycota	*Sordariomycetes*	Diaporthales	Valsaceae	Phomopsis
			0.027	(-)	Ascomycota	*Sordariomycetes*	Diaporthales	Melanconidaceae	unclassified
			0.021	(-)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.018	(-)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	unclassified
		Rhodocollybia	0.015	(+)	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	unclassified
			0.008	(+)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.007	(-)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	unclassified
			0.006	(+)	Ascomycota	Leotiomycetes	unclassified	unclassified	unclassified
		Gymnopus	0.008	(-)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.005	(+)	Ascomycota	Dothideomycetes	Pleosporales	unclassified	unclassified
			0.005	(+)	Ascomycota	Leotiomycetes	unclassified	unclassified	unclassified
			0.005	(+)	Ascomycota	Leotiomycetes	unclassified	unclassified	unclassified
		Mycena	0.041	(+)	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	unclassified
			0.022	(-)	Ascomycota	Sordariomycetes	Diaporthales	Melanconidaceae	unclassified

Table S4.2 continued

Time	Litter Type	Inocula	Contributing Difference	Abund	Phylum	Class	Order	Family	Genus
1 mo	Maple	Mycena	0.021	(-)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	unclassified
			0.012	(-)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
		Lachnocladiaceae	0.017	(+)	Basidiomycota	Agaricomycetes	Polyporales	Lachnocladiaceae	unclassified
			0.014	(-)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	unclassified
			0.013	(-)	Ascomycota	Sordariomycetes	Diaporthales	Melanconidaceae	unclassified
			0.010	(-)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
	Oak	Aspergillus	0.024	(+)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	Aspergillus
			0.019	(-)	unclassified	unclassified	unclassified	unclassified	unclassified
			0.012	(-)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.011	(-)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	unclassified
		Phomopsis	0.029	(+)	Ascomycota	Sordariomycetes	Diaporthales	Valsaceae	Phomopsis
			0.020	(-)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.019	(-)	unclassified	unclassified	unclassified	unclassified	unclassified
			0.016	(-)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
		Rhodocollybia	0.020	(-)	unclassified	unclassified	unclassified	unclassified	unclassified
			0.012	(+)	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	unclassified
			0.007	(+)	Basidiomycota	Tremellomycetes	Tremellales	unclassified	unclassified
			0.006	(-)	unclassified	unclassified	unclassified	unclassified	unclassified
		Gymnopus	0.042	(+)	Basidiomycota	Agaricomycetes	Agaricales	Pleurotaceae	Pleurotus
			0.027	(-)	unclassified	unclassified	unclassified	unclassified	unclassified
			0.023	(-)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.018	(-)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
		Mycena	0.032	(+)	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	unclassified
			0.022	(-)	unclassified	unclassified	unclassified	unclassified	unclassified
			0.012	(-)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea

Table S4.2 continued

Time	Litter Type	Inocula	Contributing Difference	Abund	Phylum	Class	Order	Family	Genus
1 mo	Oak	Mycena	0.010	(-)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
		Lachnocladiaceae	0.028	(+)	asidiomycota	Agaricomycetes	Polyporales	Lachnocladiaceae	unclassified
			0.024	(-)	unclassified	unclassified	unclassified	unclassified	unclassified
			0.018	(-)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.018	(+)	Basidiomycota	Agaricomycetes	Polyporales	Lachnocladiaceae	unclassified
3 mo	Maple	Aspergillus	0.017	(+)	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	unclassified
			0.016	(+)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.014	(-)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.013	(+)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
		Phomopsis	0.012	(-)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	Chromocleista
			0.012	(-)	Ascomycota	Sordariomycetes	Diaporthales	Melanconidaceae	unclassified
			0.010	(+)	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	unclassified
			0.010	(+)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
		Rhodocollybia	0.013	(+)	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	unclassified
			0.011	(+)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.010	(+)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.009	(+)	Ascomycota	Leotiomycetes	unclassified	unclassified	unclassified
		Gymnopus	0.019	(-)	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	unclassified
			0.016	(+)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	unclassified
			0.013	(+)	Ascomycota	Leotiomycetes	unclassified	unclassified	unclassified
			0.012	(-)	Ascomycota	Sordariomycetes	Diaporthales	Melanconidaceae	unclassified
		Mycena	0.015	(+)	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	unclassified
			0.012	(+)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.011	(-)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	unclassified
			0.011	(+)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea

Table S4.2 continued

Time	Litter Type	Inocula	Contributing Difference	Abund	Phylum	Class	Order	Family	Genus
3 mo	Maple	Lachnocladiaceae	0.012	(+)	Ascomycota	Sordariomycetes	Diaporthales	Melanconidaceae	unclassified
			0.010	(-)	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	unclassified
			0.008	(-)	Ascomycota	Leotiomycetes	Leotiomycetes_incertae_cedis	;Myxotrichaceae	Myxotrichum
			0.008	(-)	Ascomycota	Leotiomycetes	unclassified	unclassified	unclassified
	Oak	Aspergillus	0.019	(+)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.018	(-)	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	unclassified
			0.016	(-)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.014	(+)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	unclassified
		Phomopsis	0.012	(-)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.012	(-)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.012	(-)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	unclassified
			0.011	(-)	Basidiomycota	Agaricomycetes	Polyporales	Lachnocladiaceae	unclassified
		Rhodocollybia	0.020	(-)	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	unclassified
			0.016	(-)	unclassified	unclassified	unclassified	unclassified	unclassified
			0.014	(+)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.014	(+)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	unclassified
		Gymnopus	0.018	(-)	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	unclassified
			0.015	(-)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	unclassified
			0.013	(+)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.011	(+)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
		Mycena	0.014	(-)	unclassified	unclassified	unclassified	unclassified	unclassified
			0.012	(-)	unclassified	unclassified	unclassified	unclassified	unclassified
			0.011	(+)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.011	(+)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	unclassified

Table S4.2 continued

Time	Litter Type	Inocula	Contributing Difference	Abund	Phylum	Class	Order	Family	Genus
3 mo	Oak	Lachnocladiaceae	0.012	(-)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.011	(+)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.011	(+)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	unclassified
			0.009	(-)	unclassified	unclassified	unclassified	unclassified	unclassified
8 mo	Maple	Aspergillus	0.014	(-)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.008	(-)	Ascomycota	Sordariomycetes	Diaporthales	Melanconidaceae	unclassified
			0.007	(-)	Ascomycota	Leotiomycetes	unclassified	unclassified	unclassified
			0.006	(-)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	unclassified
		Phomopsis	0.018	(-)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.013	(-)	Ascomycota	Sordariomycetes	Diaporthales	Melanconidaceae	unclassified
			0.009	(-)	Ascomycota	Leotiomycetes	unclassified	unclassified	unclassified
			0.009	(-)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	unclassified
		Rhodocollybia	0.008	(+)	Ascomycota	Sordariomycetes	Ophiostomatales	Ophiostomataceae	Ophiostoma
			0.007	(+)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.007	(-)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	unclassified
			0.006	(-)	Ascomycota	Leotiomycetes	unclassified	unclassified	unclassified
		Gymnopus	0.007	(+)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.007	(+)	Ascomycota	Sordariomycetes	Ophiostomatales	Ophiostomataceae	Ophiostoma
			0.005	(+)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.005	(-)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	unclassified
		Mycena	0.034	(+)	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	unclassified
			0.019	(-)	Ascomycota	Leotiomycetes	unclassified	unclassified	unclassified
			0.014	(-)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	unclassified

Table S4.2 continued

Time	Litter Type	Inocula	Contributing Difference	Abund	Phylum	Class	Order	Family	Genus
8 mo	Maple	Mycena	0.013	(-)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	unclassified
		Lachnocladiaceae	0.019	(-)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	unclassified
			0.016	(+)	Ascomycota	Leotiomycetes	Helotiales	unclassified	unclassified
			0.011	(-)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	unclassified
			0.010	(+)	Ascomycota	Leotiomycetes	Leotiomycetes_incertae_sedis	Myxotrichaceae	Myxotrichum
	Oak	Aspergillus	0.010	(+)	Ascomycota	Sordariomycetes	Ophiostomatales	Ophiostomataceae	Ophiostoma
			0.009	(+)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	Aspergillus
			0.008	(-)	Ascomycota	Sordariomycetes	Calosphaeriales	Calosphaeriaceae	Togninia
			0.008	(+)	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	unclassified
		Phomopsis	0.017	(-)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.012	(+)	Ascomycota	Sordariomycetes	Diaporthales	Valsaceae	Phomopsis
			0.009	(+)	Ascomycota	Sordariomycetes	unclassified	unclassified	unclassified
			0.009	(-)	Ascomycota	Sordariomycetes	Diaporthales	Melanconidaceae	unclassified
		Rhodocollybia	0.007	(-)	unclassified	unclassified	unclassified	unclassified	unclassified
			0.007	(-)	Ascomycota	Sordariomycetes	Calosphaeriales	Calosphaeriaceae	Togninia
			0.007	(-)	Ascomycota	Sordariomycetes	Hypocreales	Bionectriaceae	unclassified
			0.006	(-)	Ascomycota	*Eurotiomycetes*	Eurotiales	Trichocomaceae	unclassified
		Gymnopus	0.019	(-)	Ascomycota	Dothideomycetes	Pleosporales	unclassified	unclassified
			0.018	(-)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.016	(+)	Basidiomycota	Agaricomycetes	Agaricales	Pleurotaceae	Pleurotus
			0.012	(-)	Ascomycota	Leotiomycetes	Helotiales	unclassified	unclassified

Table S4.2 continued

Time	Litter Type	Inocula	Contributing Difference	Abund	Phylum	Class	Order	Family	Genus
8 mo	Oak	Mycena	0.037	(+)	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	unclassified
			0.025	(-)	Ascomycota	Dothideomycetes	Pleosporales	unclassified	unclassified
			0.020	(-)	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea
			0.017	(-)	Ascomycota	Leotiomycetes	Helotiales	unclassified	unclassified
		Lachnocladiaceae	0.018	(-)	Ascomycota	Dothideomycetes	Pleosporales	unclassified	unclassified
			0.013	(+)	Ascomycota	Leotiomycetes	Helotiales	unclassified	unclassified
			0.011	(+)	Ascomycota	Sordariomycetes	Hypocreales	unclassified	unclassified
			0.011	(+)	Ascomycota	Leotiomycetes	Leotiomycetes incertae_sedis	Myxotrichaceae	Myxotrichum

Table S4.3. Cumulative litter community respiration in each treatment combination (n = 10) through time, calculated using the sigmoidal Gompertz model ($y(t) = A\times \exp[-\exp\left(\frac{u-e}{A}(\lambda - t) + 1\right)]$). Length of lag time is represented by λ, while μ indicates maximum rate of respiration, and A estimates the amount of substrates available for metabolism. Multiple comparisons were conducted using a protected Tukey's HSD. Statistical significance is denoted according to departures from the control community.

Treatment	λ (d)	μ (mg g^{-1} d^{-1})	A (mg g^{-1})
I. Maple			
Control	16.8 (0.63)	1.17 (0.10)	69.20 (3.21)
Aspergillus	16.3 (0.34)	0.96 (0.07)	70.30 (3.69)
Phomopsis	9.6 (0.91) ***	0.97 (0.03)	65.81 (2.29)
Rhodocollybia	16.4 (0.99)	1.13 (0.06)	65.86 (2.06)
Gymnopus	16.8 (0.78)	1.26 (0.05)	76.55 (2.65)
Mycena	18.3 (0.42)	1.48 (0.07)	101.44 (6.43)***[1]
Lachnocladiaceae	13.2 (0.79)	1.49 (0.12)	91.93 (8.76)
II. Red Oak			
Control	14.3 (0.82)	0.67 (0.03)	46.52 (2.07)
Aspergillus	13.2 (0.92)	0.58 (0.03)	51.09 (1.72)
Phomopsis	6.4 (0.60) ***	0.72 (0.03)	55.17 (2.41)
Rhodocollybia	13.6 (0.85)	0.67 (0.05)	47.32 (2.42)
Gymnopus	16.2 (1.58)	1.40 (0.12)***	106.15 (16.85)***
Mycena	18.0 (0.84)	1.09 (0.06)*	90.71 (5.08)***
Lachnocladiaceae	13.0 (0.85)	1.24 (0.06)***	81.07 (5.7)***

[1] Relative to the control treatment , *** represents significance at $\alpha < 0.01$, ** denotes $\alpha < 0.05$, * denotes $\alpha < 0.10$

CHAPTER V

Conclusions

Despite the unique characteristics of microorganisms and their previous exclusion from traditional ecological principles, my dissertation reveals that the ecological forces structuring plant and animal communities also shape the assembly of soil communities composed of saprotrophic fungi. For example, dispersal limitation and ecological drift were important forces structuring fungal communities (Chapter III), irrespective of the small size of fungi and potential for long-distance spore dispersal (Hallenberg and Kuffer 2001, Favet et al. 2012). Functional traits also appeared to shape competitive dynamics of fungal communities, despite support for the functional equivalence of soil microbial communities (Talbot et al. 2014). In support of this assertion, changes in resource availability during plant secondary succession predictably drove the turnover of fungal communities and their physiological capacities to degrade plant detritus (Chapter II). In addition, the traits of early fungal colonists contributed to the strength of priority effects during community assembly (Chapter IV). Together, the importance of selection, dispersal limitation and priority effects in structuring microbial communities reveals that the fundamental mechanisms governing the most abundant and diverse organisms on Earth are not distinct from other organisms. Furthermore, the observed link between fungal community composition and their functional traits indicates that the mechanisms of fungal community assembly are ecologically significant. For example, fungal functional traits changed

concomitantly with community composition during plant secondary succession (Chapter II) as well as following initial colonization of an empty niche (Chapter IV), demonstrating the important consequences of selection and priority effects on the functional potential of the fungal community. By dispelling prior misconceptions of ubiquitous dispersal and functional redundancy, my work strengthens the call for a single framework with the breadth and flexibility to investigate the processes that structure all biota, especially diverse communities of fungi that inhabit soil (Vellend 2010, Nemergut et al. 2013).

Armed with a synthetic framework of microbial community assembly, there is a need to move beyond correlative observations in order to gain mechanistic insights into factors that structure microbial composition and function (Prosser et al. 2007, Nemergut et al. 2013). A trait-based approach is a powerful method to elucidate the roles of selection, dispersal, drift and diversification across local, regional, and global scales at time steps ranging from hours to millenia (McGill et al. 2006, Allison 2012, Crowther et al. 2014). For example, there is a need to understand the fungal traits that determine fitness differences at the local scale, such as growth rate, foraging morphology, and the production of toxic secondary metabolites (Crowther et al. 2014, Aguilar-Trigueros et al. 2015). Additionally, we do not understand the tradeoffs contributing to the distribution of soil microbial communities at local versus regional scales, which may include morphological traits enhancing long-distance dispersal (*i.e.*, spore size and production), tolerance to environmental gradients, and physiological mechanisms regulating dormancy (Lennon and Jones 2011, Norros et al. 2014, Aguilar-Trigueros et al. 2015). Furthermore, it is necessary to understand the conditions that lead to lasting effects of historical contingencies on present-day assemblages. For example, strong priority effects may result from communities comprised of organisms with low rates of dispersal, rapid establishment, and strong

capacities to modify habitats (Chase 2003, Vannette and Fukami 2014), thereby weakening contemporary selection relative to historical processes of community assembly.

Due to the utility of molecular characterization, the field of microbial ecology is uniquely positioned to employ trait-based approaches to study community assembly. Despite years of methodological limitations, metagenomics, meta-transcriptomics and meta-proteomics are emerging tools that enable microbial ecologists to investigate linkages between taxonomic, phylogenetic, and functional traits of the community as a whole (Baldrian et al. 2012, Schneider et al. 2012). For example, these 'omics techniques can inform how evolutionary history of microorganisms contributes to ecological differences structuring distribution patterns across a landscape (Cadotte et al. 2013). Because community-wide metrics cannot reveal detailed information about biotic interactions (McGill et al. 2006, Crowther et al. 2014), individual-based approaches must be used in concert to characterize important life-history tradeoffs through a focus on the 'autecology' of important organisms (Peay 2014). This information will increase understanding of the genomic features associated with ecological differences between species that, in turn, impact the outcome of biotic interactions.

As mediators of C and N cycling in soils, understanding the functional implications of microbial community assembly is of ecosystem-scale significance. My work presents evidence that the ecological factors structuring saprotrophic fungal communities have direct consequences for the potential of the soil microbial community to degrade plant detritus. Despite this important insight, it is necessary to gain a clear understanding of the molecular mechanisms by which microbial traits are expressed, translated, and ultimately impact biogeochemical processes. A single framework of community assembly combined with a functional trait-focused approach provides microbial ecologists with the opportunity to advance our ecological understanding of

the processes and ecological implications that underlie the distribution and abundance of microorganisms across spatial and temporal gradients.

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